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SURVIVAL AND TRANSPORT OF PATHOGENS IN SLUDGE-AMENDED SOIL

A Critical Literature Review

by

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Austin, Texas 78712

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16. ABSTRACT

A study was undertaken to critically review available information on the survival and transport of pathogens from municipal wastewater sludges applied to land. Unfortunately, the amount of quantitative, comparable data related to pathogen behavior in sludge-amended soils is limited. Most available data are restricted to Salmonella and indicator bacteria.

In general, Salmonella showed a 90% (T_{90}) reduction within 3 weeks in sludge-amended soils. In warm climates, inactivation of viruses near the surface was quite rapid with a median T_{90} of 3 days, while comparable inactivation at low temperatures required approximately 30 days. Maximal parasite survival, as represented by Ascaris, was relatively long with a median T_{90} of 77 days.

Extremely limited vertical movement of some pathogens may be anticipated in sludge-amended soils. Although monitoring at sludge application sites has not revealed any impact on the bacterial quality of groundwater, limited transport of indicator bacteria to depths up to 180 cm has been reported. Under field conditions including exposure to natural rainfall, virtually no viruses have been detected in soil-water percolates. Available literature strongly favors the contention that parasitic ova are retained at the point of sludge introduction to the soil. Finally, insufficient data are available to provide the various factors required to adequately model pathogen survival and transport in sludge-amended soils.

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water systems. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxic Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Research Laboratory is that component of EPA's Research and Development program concerned with preventing, treating, and managing municipal and industrial wastewater discharges; establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product uses. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

The use of municipal wastewater sludges for application to the land is increasing. Current laws and regulations on the one hand encourage this use and on the other hand define criteria for safe use, thereby limiting the practice. Since there is little question as to the presence of pathogens in municipal wastewater sludges, documentation of their fate in sludge applied to land is required to adequately manage these wastewater residuals and to formulate scientifically based regulations.

Francis T. Mayo, Director
Water Engineering Research Laboratory

ABSTRACT

A study was undertaken to critically review available information on the survival and transport of pathogens from municipal wastewater sludges applied to land through amending soils. Specifically, attempts were made to describe the value of past work related to the topic and to identify those areas where inadequate information exists to predict pathogen survival and transport.

The study involved acquiring more than 800 abstracts and 200 documents, either from the literature or directly from researchers. Of these, 67 documents were judged to contain usable information based on the guidelines developed for the literature evaluation.

Overall, the amount of quantitative, comparable data related to pathogen survival or transport in sludge-amended soils is extremely limited. Most of the available data are restricted to *Salmonella* and indicator organisms, with even fewer data available for viruses and parasites.

Of the physical and meteorological parameters, only temperature could be related to microorganism survival in sludge-amended soils. In general, survival increased as temperature decreased. The one exception involved fecal coliforms, which increased with increasing temperature at depths of 5 to 15 cm. This anomaly was attributed to the enriched environment of sludge-amended soils and the protection that depth afforded the organisms from other environmental stresses. Unfortunately, all of these data were derived from a single study.

In general, *Salmonella* showed a 90% reduction within 3 weeks in sludge-amended soils. In warm climates, inactivation of viruses near the surface was quite rapid. The median T_{90} was 3 days, and the median T_{99} was 6 days. However, at low temperatures T_{90} values of approximately 30 days were observed for viruses. Parasite survival was relatively long near the surface when compared with both *Salmonella* and viruses. The median T_{90} was 77 days. Insufficient data exist to evaluate parasite survival at greater depths.

Available literature suggests that as long as bacteria remain viable, they are subject to transport in runoff. Similarly, parasitic ova have been recovered from irrigation return flow at a sludge application site.

Extremely limited vertical movement of some pathogens may be anticipated in sludge-amended soils. Although monitoring at sludge application sites has not revealed any impact on the bacterial quality of groundwater, limited transport to a depth of 180 cm has been reported. Under field conditions, including exposure to natural rainfall, virtually no viruses have been detected in soil-water percolates. This result is probably related to the observation that viruses are relatively tightly bound to sludge. Finally, available literature strongly favors the contention that parasitic ova are retained at the point of sludge introduction to the soil. Transport to groundwater seems unlikely given the relative size of most parasitic forms.

Unfortunately, insufficient data are available to adequately model pathogen survival and transport in sludge-amended soils. This situation can be rectified through the proper design and initiation of appropriate studies.

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SECTION 1

INTRODUCTION

The development of research programs and regulations in a particular area depends on the aggregate knowledge of both the existence and the value of prior research. Clearly, this type of information is not always readily available. In fact, a great deal of effort is often required to develop the basic information, evaluate the data, and determine its value under a given set of conditions.

The application of municipal wastewater sludges to land is increasing in popularity, and the practice is likely to expand rapidly in the years to come. Thus this practice needs to be regulated with regard to pathogens known to be present in sludges. While regulations should be founded on a sound scientific base, unified documentation of the fate of pathogens in sludge applied to land is not available.

Interim regulations relating to sludge treatment and disposal have been developed and were published in the Federal Register in September 1979. They appear in the current Code of Federal Regulations (CFR, Title 40, "Criteria for Classification of Solid Waste Disposal Facilities and Practices," par. 257.3-6, Disease, Interim Final Regulations, effective October 15, 1979).

The CFR criteria allow the following three degrees of sludge disposal restriction depending on the degree of pathogen reduction:

1. Raw sludge cannot be applied to, incorporated into, or injected shallowly into the land surface. The basis for this requirement is evidently the potential hazard from the pathogens and also the need to minimize attraction of disease vectors (insects, rodents) by sludge deposited on or leaking to the land surface.

2. Sludge whose pathogen content has been reduced significantly (see definition below) can be applied to or injected shallowly into the land surface subject to several restrictions: public access is controlled for at least 12 months, grazing by animals whose products are consumed by humans is prevented for 1 month, and crops grown for human consumption that contact the sludge are not grown within 18 months subsequent to sludge application or incorporation. The 18-month interdiction is not required if the edible portion of the crop does not contact the sludge.

3. Sludge whose pathogen content has been further reduced (see definition below) is not required to meet any other requirements under the CFR criteria.

The definitions of significant reduction and further reduction of pathogens are vitally important to the application of the criteria. For a sludge treatment process to qualify as a process to significantly reduce pathogens (PSRP), it must produce a pathogen reduction at least equivalent to that obtained by good anaerobic digestion. The logic of the definition rests on the observation that agricultural use of anaerobically digested sludge as a fertilizer has been practiced for many years with no evidence that the practice has caused human illness, provided the digestion is adequate. Since these farming operations have been on land with limited access and clearly defined use, this same restriction was applied to the use of PSRP sludge.

Processes to further reduce pathogens (PFRP) include composting, pasteurization, and treatment by electron beams or gamma ray irradiation. A new process must perform in a similar way to these processes. Their common feature is that, when properly operated, they expose sludge to conditions that would essentially eliminate pathogens of concern.

Unfortunately, neither these definitions nor their application appear to have been based on scientific information related to the survival and transport of pathogens in sludge-amended soils. Rather, it appears that they are based on the expected operational performance of specific unit processes and the absence of health effects directly related to land application practices. However, the paucity of documented health problems associated with the land application of sludges may reflect the lack of sufficiently sensitive epidemiological tools to detect small-scale incidents of disease (Gerba, 1983).

Little question exists about the need for regulating the land application of municipal wastewater sludges with regard to known pathogens in these sludges. However, these regulations should be founded on both a complete understanding of the fate and transport of pathogens in sludge-amended soils and the epidemiological implication associated with the numbers of organisms to which humans are subjected as a result of these practices. Clearly, this approach requires the documentation of the fate of pathogens in sludge when applied to land.

SECTION 2

CONCLUSIONS

1. The amount of quantitative, comparable data describing pathogen survival or transport in sludge-amended soils is extremely limited. Survival data is available only for Salmonella, selected enteric viruses, and Ascaris ova while studies on pathogen transport are limited to Yersinia and certain viruses.

2. Much of the available literature records the presence (persistence) or absence of microorganisms, but does not allow an assessment of inactivation.

3. Where adequate quantitative data exist, these observations can be made:

   - Inactivation of indicator bacteria as described by median T_{90} values was greater than that observed for Salmonella.
   - Viral inactivation appears to be faster than Salmonella near the soil surface. However, all but one study used for the estimation of viral die-off were conducted under rather narrow temperature ranges, thus highlighting a potential bias in the application of these values.
   - Inactivation of parasites near the soil surface is relatively slow, perhaps as much as 5 times slower than Salmonella and more than 13 times slower than viruses.

4. Exceedingly long survival times sometimes cited for Salmonella arise from studies in which high levels of added organisms (10^6 - 10^{10}/L) were present.

5. The only strong evidence for bacterial regrowth in sludge-amended soils is related to organisms of the coliform group.

6. Of the physical and meteorological paramaters considered, only temperature could be correlated with microorganism survival. Increasing temperature was observed to hasten die-off near the surface, while at depths of 5-15 cm fecal coliform levels increased as temperatures increased. Once again, the limited amount of published data may be biasing this correlation, as all of the results for fecal coliform survival at depths greater than 5 cm were taken from a single study.

7. Inadequate data exist to assess critically the vertical transport of pathogens from sludge-amended soils. However, several general observations can be made:

- o Data collected at all but one operational field site have not demonstrated a deterioration of groundwater quality related to sludge application.
- o Selected studies have documented limited bacterial movement to depths of 180 cm beneath sludge-amended soil.
- o Laboratory studies suggest the viral retention is enhanced in sludge-amended soils relative to effluent-irrigated soils.
- o The size of parasites appears to preclude their vertical movement from sludge-amended soils, although studies designed for this purpose were not found.

8. Exceedingly few studies have addressed the issue of microorganisms in runoff from sludge-amended soils. However, there is a high probability that uncontrolled runoff will contain pathogens as long as viable organisms are present in sludge-amended soils.

9. Insufficient data are available to adequately model pathogen survival or transport in sludge-amended soils. Not only are microbial results limited, but prevailing environmental and soil conditions have not been adequately documented in many published reports.

SECTION 3

RECOMMENDATIONS

The development of regulations designed to protect the public from pathogens in municipal wastewater sludges in sludge-amended soils requires an understanding of four major issues:

1. The levels of specific pathogens of major public health significance contained in municipal wastewater sludges;

2. The ability of specific sludge treatment unit processes to reduce the concentrations of these pathogens;

3. The survival and/or transport of these pathogens in sludge-amended soils; and

4. The risk to humans ingesting a specific number of these pathogens.

While there may be some debate as to the state of knowledge related to issues 1, 2 and 4, the objectives of this study were focused on issue 3 only. Further, it is clear that existing data do not permit a complete understanding of this issue.

Thus, the following specific recommendations are designed to obtain the required data:

1. Studies specifically designed to develop comprehensive data relative to the survival and transport of specific pathogens in sludge-amended soils should be conducted.

2. These studies should be restricted to representative pathogens such as *Salmonella*, human enteric viruses, and parasites indigenous to municipal wastewater sludges.

3. Critical parameters that should be evaluated include temperature, moisture, sludge loading, and soil type.

4. While it would be desirable to conduct these studies under field conditions with indigenous organisms, the sensitivity of currently employed detection methodologies and the existence of a wide variety of uncontrolled variables may limit this approach.

5. The use of selected seeded organisms or sludges with high levels of representative pathogens under closely controlled laboratory conditions may be the most reasonable approach.

6. Laboratory experimentation must be carefully designed to simulate a range of temperature, moisture, and sludge loading conditions found in the field nationwide.

SECTION 4

BACKGROUND

In the nineteenth century, it became relatively common for municipalities to operate sewage farms. Some of those facilities were successful as measured by such standards as absence of excessive odors or standing water. Eventually, as urban expansion encroached on available land and technological interests turned to the design of sewage treatment plants, most sewage farms were abandoned. However, land application as a waste-management alternative was reemphasized as a direct result of the enactment of PL 92-500 in 1972.

Today, municipal wastewater sludge management is perhaps one of the most visible and complex problems facing those responsible for wastewater treatment facilities. The combination of greatly increased sludge volumes and the narrowing of formerly used disposal options (such as ocean dumping) compounds this problem.

MANAGEMENT OF MUNICIPAL WASTEWATER SLUDGE

An integral part of almost any wastewater treatment plant is the sludge management system. Residual solids are produced in nearly every unit process associated with conventional wastewater treatment. Approximately 6 million dry metric tons of municipal wastewater sludges are generated annually in the United States. By the year 2000, the quantity of municipal wastewater sludge produced is expected to almost double (USEPA, 1984). The increase will be a direct result of upgrading existing treatment plants as well as building and operating new facilities necessary to adequately treat increasing volumes of municipal wastewater prior to discharge or reuse.

Sludge management currently accounts for approximately one-half of the cost of wastewater treatment (USEPA, 1984). Thus, a major goal is to reduce the costs of sludge management. A further goal is to reduce to an acceptable level the risks to public health, safety and welfare that arise from and are otherwise associated with sludge disposal.

Sludge disposal alternatives include three main options:

1. Incineration (the ash must be disposed of elsewhere),

2. Ocean dumping, or

3. Land disposal (either in landfills or application to land).

Current regulations restrict the discharge of sludge into the ocean. This important change has resulted in an increased emphasis on the land disposal/application option.

LAND DISPOSAL OF SLUDGE

Land disposal of sludge can be accomplished by a number of methods, including direct deposition on the surface with or without incorporation into soil, subsurface injection, and sanitary landfills. The sludge land application/disposal options are:

1. Agriculture: Use of sludge as a source of fertilizer nutrients or as a soil amendment to enhance crop production.

2. Silviculture: Use of sludge on forested land to enhance forest productivity.

3. Land Reclamation: Application of sludge to strip-mined lands, mine tailings, or other disturbed or marginal land for the purpose of revegetation and reclamation.

4. Dedicated Land Disposal: Application of sludge to soils, with or without vegetation, for the primary purpose of sludge disposal.

There are a large number of pathways whereby contaminants in sludge can affect humans. Generally, these pathways involve ingestion, inhalation, entry through abrasions in the skin, entry through orifices other than the nose or mouth, and diffusion through the pores of the skin. Of primary importance in the non-occupational exposure associated with the land application of sludges are ingestion and inhalation (Sorber and Moore, 1985).

MICROBIOLOGICAL HEALTH ASPECTS OF LAND APPLICATION

The concentration of pathogens in wastewater and thus in wastewater sludges is influenced by a number of factors, including the age and health of the contributing population, population density, sanitary habits, and the season of the year. The microorganisms of public health concern are generally classified into three broad categories: bacteria, viruses, and parasites. Parasites are often further differentiated into helminths and protozoans. There are hundreds of organisms that fall into these various categories and that may be present in domestic wastewaters.

Available detailed reviews describe a wide variety of disease-causing microorganisms which potentially may be transmitted through environmental exposure (Akin et al, 1978; Sobsey and Olson, 1983; Bitton and Gerba, 1984; Gerba, 1983). Generally, such lists include most infectious agents known to be transmitted by the fecal-oral route. A more focused list of microbial agents can be prepared, however, if additional criteria such as demonstrable presence in wastewaters or sludges and/or documented environmental transmission of disease are applied. Table 1 provides such a listing. Note that while some of the organisms listed are overt pathogens, reports of their occurrence in wastewater and sludges in the United States are quite rare.

TABLE 1. ORGANISMS OF MAJOR CONCERN IN LAND APPLICATION OF MUNICIPAL WASTEWATER AND SLUDGES (After Sorber and Moore, 1985)

Group and Name of Organism		Primary Disease	Remarks
Bacteria	*Legionella pneumophila*	Acute respiratory disease	Aerosol transmission documented, but no cases linked to wastewater exposure to date
	Salmonella sp.	Gastroenteritis, typhoid and paratyphoid fever	
	Shigella sp.	Bacillary dysentery	Overt pathogens but low probability of occurrence in wastewater in the United States.
	Vibrio cholerae	Cholera	
Viruses	Hepatitis A virus	Infectious hepatitis	Documented waterborne transmission
	Non-A, Non-B hepatitis	Hepatitis	Preliminary evidence for waterborne transmission
	Norwalk-like agents	Gastroenteritis	Documented waterborne transmission
	Rotavirus	Gastroenteritis	Documented waterborne transmission
Helminths	*Ascaris* sp.	Ascariasis	
Protozoans	*Giardia lamblia*	Giardiasis (gastroenteritis)	Documented waterborne transmission

However, it should also be understood that advances in microbiological and medical sciences will identify additional pathogenic organisms linked to environmental disease transmission in the future.

The concentrations of some of these organisms isolated from raw wastewater are shown in Table 2. Similarly, concentrations of these organisms found in raw municipal wastewater sludges are shown in Table 3. Ranges are a function of the population demography as well as regional and social issues such as water use in the home and sanitary practices.

TABLE 2. RANGES OF SELECTED ORGANISMS FOUND IN RAW MUNICIPAL WASTEWATER

Organism	Concentration (Organisms/100 ml)	Reference
Salmonella sp.	$10^2 - 10^6$	Foster and Englebrecht, 1973
Shigella sp.	$<10^0 - \geq 10^2$	Camann et al, 1986
Total Enteroviruses	$10^1 - 10^3$	Gerba, 1983; Camann et al, 1986
Ascaris sp.	$10^0 - 10^1$	Larkin et al, 1978
Giardia lamblia	$10^2 - 10^3$*	Jakubowski, 1984

* Estimated

TABLE 3. RANGES OF SELECTED ORGANISMS FOUND IN UNTREATED MUNICIPAL WASTEWATER SLUDGES

Organism	Concentration (Organisms/g dry weight)	Reference
Salmonella sp.	$10^2 - 10^3$	Ward et al, 1984
Shigella sp.	$<10^1 - >10^1$	Harding et al, 1980
Viruses	$10^2 - 10^4$	Ward et al, 1984
Ascaris sp.	$10^1 - 10^3$	Reimers et al, 1981
Giardia lamblia	Unknown*	

*Limited data available; however, low concentrations expected.

Bacteria

During 1983 over 44,000 cases of salmonellosis and almost 20,000 cases of shigellosis in the United States were reported to the Centers for Disease Control (CDC) (1984). This is contrasted with one case of cholera and 852 cases of legionellosis reported during the same period. While such cumulative data highlight the relative prevalence of these diseases in the United States, the routes of infection are seldom documented. Presumably, few if any of these cases were associated with wastewater. Nevertheless, both *Salmonella* and *Shigella* species remain very important in the United States. Generally, pathogenic bacteria are fairly stable in raw wastewater especially during the relatively short time required to reach the treatment works. Further, there have been reports of replication of some species in wastewater sludges (Yeager and Ward, 1981).

Viruses

During 1983 more than 21,500 cases of hepatitis A were reported to CDC (1984). A limited number of cases were known to be waterborne as were cases of Norwalk-type viral gastroenteritis and rotavirus gastroenteritis (Sobsey and Olson, 1983). Further, almost 3,500 cases of non-A, non-B hepatitis were reported to CDC in 1983 (1984). While there have been no documented cases of environmental transmission of this agent in the United States, such transmission has been reported in foreign countries (Nouasria et al, 1984; Tabor, 1985).

While certain human viruses have been shown to be fairly stable in raw wastewater, they cannot replicate outside a susceptible host. Thus, their numbers will decrease with time as they are exposed to an unfavorable environment. It has been shown, however, that protection from inactivation can be afforded by viral adsorption or occlusion in solids.

Parasites

Most of the helminths in wastewater and sludges are only incidentally considered a potential problem to humans in most areas of the United States. More often than not, they are involved in the infection of animals. Nevertheless, *Ascaris* is considered to be the most important of the helminths and *Giardia lamblia*, the most important protozoan as far as infection of humans in the United States is concerned. *G. lamblia* is particularly important in those areas where drinking water outbreaks are common (i.e., the Pacific Northwest, the Rocky Mountain region, and the higher elevations of New England).

WASTEWATER TREATMENT

Wastewater treatment affects the various organism types in different ways. In general, however, it has been observed that microbial segregation occurs during conventional wastewater treatment. Bacteria and viruses tend to become associated with the sludge component as do the heavier eggs of certain parasites such as *Ascaris*. A recent report by Sykora and associates (1986) showed that in six of seven treatment plants more than 90% of *Giardia* cysts were removed from wastewater by secondary treatment. Thus, sludges may carry a large number of these protozoan cysts as well.

A typical treatment train for conventional wastewater treatment is shown in Figure 1. It should be noted that none of the processes shown (with the exception of disinfection) are designed for the reduction of pathogens. Thus, the majority of pathogen removal from wastewater is fortuitous (Sorber and Moore, 1985).

The reduction of various pathogen groups associated with typical sludge treatment processes is shown in Table 4. It can be seen that parasites are least affected by most of the processes, although composting and air drying are superior to the others. In the absence of extensive treatment, however, wastewater sludges will contain measurable concentrations of pathogenic microorganisms. The fate of these sludge-associated organisms in amended soils is the topic of this report.

TABLE 4. EFFECTIVENESS OF VARIOUS SLUDGE TREATMENT PROCESSES IN THE REDUCTION OF PATHOGENS (After Ward et al, 1984)

	Log_{10} Reductions		
Sludge Treatment Process	Bacteria	Viruses	Parasites
Mesophilic anaerobic digestion	0.5 - 4	0.5 - 2	<0.5
Aerobic digestion	0.5 - 4	0.5 - 2	<0.5
Composting	2 - >4	2 - >4	2 - >4
Air drying	0.5 - >4	0.5 - >4	0.5 - >4
Lime stabilization	2 - >4	>4	<0.5

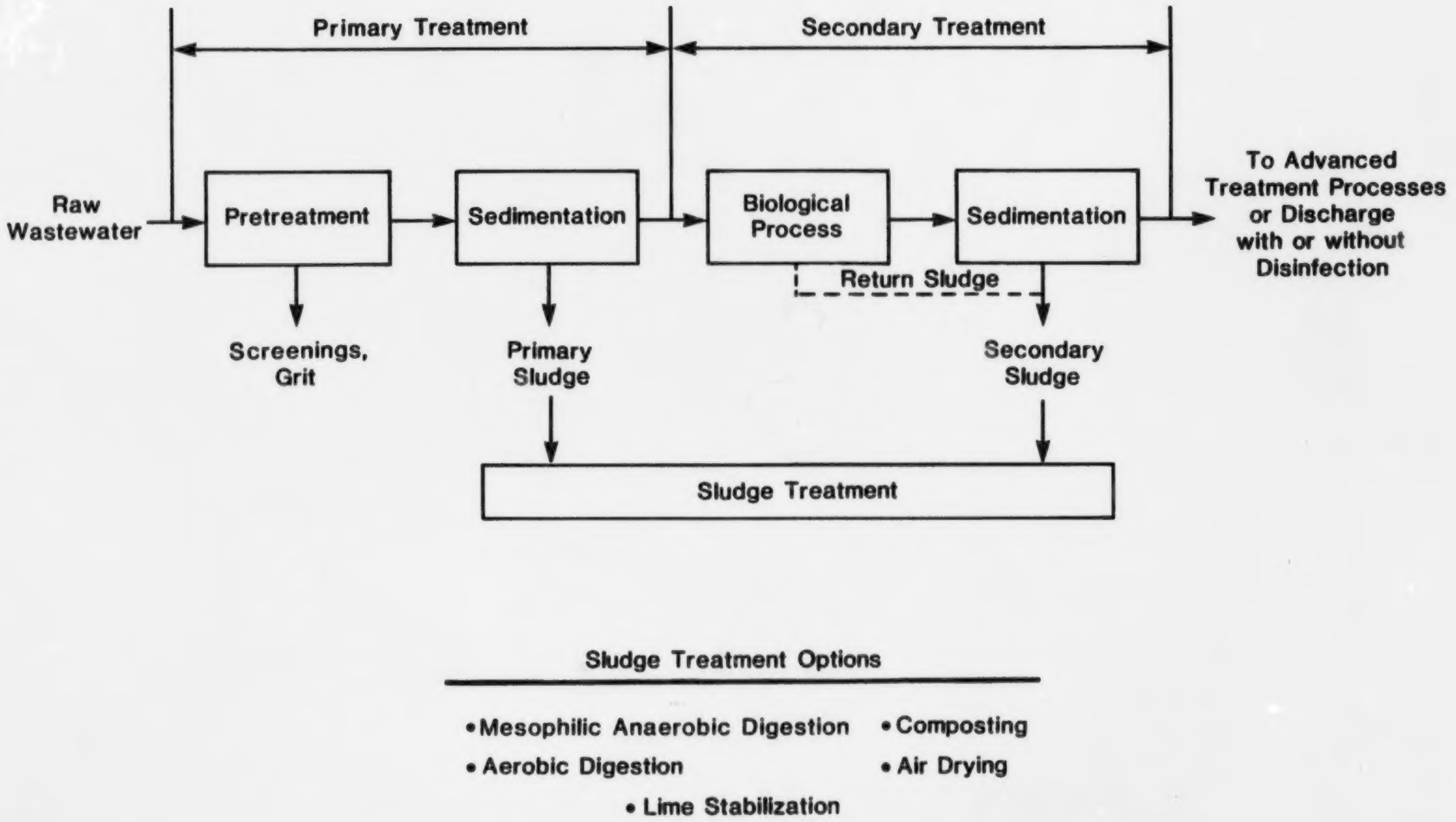
Primary Treatment
Secondary Treatment
Raw Wastewater
Pretreatment
Sedimentation
Biological Process
Sedimentation
To Advanced Treatment Processes or Discharge with or without Disinfection
Return Sludge
Screenings, Grit
Primary Sludge
Secondary Sludge
Sludge Treatment
Sludge Treatment Options
•Mesophilic Anaerobic Digestion
•Composting
•Aerobic Digestion
•Air Drying
•Lime Stabilization

Figure 1. Selected Conventional Wastewater Treatment Options

SECTION 5

OBJECTIVES

The overall objective of this study was to critically review available information relative to the survival and transport of pathogens from municipal wastewater sludges when applied to the land through amending soils. Specific objectives included the following:

1. Conduct primary literature searches (titles and abstracts);
2. Secure hard copies of documents that hold promise as contributing to the study;
3. Conduct secondary literature searches and document retrieval;
4. Compile a comprehensive bibliography;
5. Critically review each document;
6. Develop a statement of the value of past work related to the subject matter; and
7. Identify those areas where inadequate information exists to predict pathogen survival and transport in sludge-amended soils.

SECTION 6

METHODS

LITERATURE SEARCHES

Extensive literature searches were conducted and a significant number of documents were accumulated from a variety of sources. The search for relevant documents was carried out in four steps: primary (data base) searches, secondary searches, author contacts, and manual searches.

Primary Literature Searches

The primary literature search scheme focused on developing a sequence of keywords that would result in the comprehensive recovery of relevant abstracts while minimizing recovery of irrelevant material. While a spectrum of keywords was evaluated, only a limited number were considered to hold promise for reaching this objective. Various keyword sequences were tested to evaluate their performance. The use of specific pathogen group names such as viruses, parasites, or microorganisms did not prove to be an effective or efficient approach to recovery of useful material from data bases. Keywords used successfully in these trial runs included sludge, soil, transport, survival, sludge disposal, health, persist, land, and soil and their various combinations. The sequence found to be most effective was Sludge? and (Soil? or Land?) and (Transport or Survival or Persist?).

All abstracts in the primary data bases searched that explicitly used these words were recovered. For instance, to be recovered an abstract had to use the word sludge and either the word soil or land and either the word transport or survival or persist. The question marks (?) extended the recovery to all forms of the base word in the searches.

A large number of abstracts beyond the scope of this study were recovered by this broad based approach. However, it seemed more appropriate to err on the side of excess recovery rather than risk missing relevant literature.

A total of 12 primary literature data bases were searched. This resulted in the acquisition of a total of 819 titles and abstracts. Upon reading the abstracts and eliminating duplication, a net total of 95 unique documents were identified for hard-copy acquisition.

Data bases searched along with the subsequent recoveries of total and relevant abstracts are presented in Table 5. A description of each data base searched follows:*

TABLE 5. RECOVERY OF ABSTRACTS FROM DATA BASE SEARCHES

Data Base	Total Number of Abstracts Recovered	Documents Identified for Acquisition
AQUALINE, 1964-84	294	22
AQUATIC SCIENCES, 1978-84	2	0
BIOSIS PREVIEWS, 1972-84	24	11
CA SEARCH, 1972-84	16	1
COMPENDEX, 1970-84	92	22
ENVIROLINE, 1970-84	93	6
ENVIRONMENTAL BIBLIOGRAPHY, 1974-84	6	1
MEDLINE, 1973-84	16	4
NTIS, 1964-84	84	6
POLLUTION ABSTRACTS, 1970-84	28	13
WATER RESOURCES ABSTRACTS, 1968-84	150	7
WATERNET™, 1971-84	14	2
Totals	819	95

1. AQUALINE, 1964-1984, 41,000 citations, monthly updates.

AQUALINE provides access to information on every aspect of water, wastewater, and the aquatic environment. Corresponding to Water Research Center Information, AQUALINE coverage includes water resources development and management, drinking water quality, water treatment, sewage systems, sludge disposal, groundwater pollution, river management, tidal waters, quality monitoring, environmental protection. Worldwide sources cited are 600 periodicals, research reports, legislation, conference proceedings and preprints, books, monographs, pamphlets, dissertations, translations, standards and specifications, and miscellaneous publications from water-related institutions worldwide.

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*The descriptions provided herein have been extracted from the DIALOG catalog as published by Lockheed Inc., Palo Alto, CA.

2. AQUATIC SCIENCES AND FISHERIES ABSTRACTS, 1978-1984, 148,500 records, monthly updates.

AQUATIC SCIENCES AND FISHERIES ABSTRACTS (ASFA) is a comprehensive data base on life sciences of the seas and inland waters as well as related legal, political, and social topics. It includes information on aquatic biology, oceanography, fisheries, and water pollution. Corresponding to the printed publication, Aquatic Sciences and Fisheries Abstracts, Parts 1 and 2, ASFA cites primary journals and such other source documents as books, conference proceedings, and technical research reports.

3. BIOSIS PREVIEWS, 1972-1984, 3.7 million records, semi-monthly updates.

BIOSIS PREVIEWS contains citations from both Biological Abstracts and Biological Abstracts/RRM (formerly entitled Bioresearch Index), the major publications of BioSciences Information Service of Biological Abstracts. Together, these publications constitute the major English language service providing comprehensive worldwide coverage of research in the life sciences. Over 9,000 primary journals and monographs as well as symposia, reviews, preliminary reports, semi-popular journals, selected institutional and government reports, research communications, and other secondary sources provide citations on all aspects of the biosciences and medical research.

4. CA SEARCH, 1972-1984, 5.9 million records, biweekly updates.

CA SEARCH is an expanded data base which contains the basic bibliographic information appearing in the printed Chemical Abstracts. Index entries containing CA General Subject Headings from a controlled vocabulary, and the CAS Registry Numbers (a unique number assigned to each specific chemical compound) appear complete with modifying phrases. Additional uncontrolled complete keyword index phrases and cross referenced CA General Subject Headings are also included. This merged data base provides the user with many more access points to each covered citation and thus greatly increases the ease of searching for both broadly defined topics and specific compounds.

5. COMPENDEX, 1970-1984, 1.2 million records, monthly updates.

The COMPENDEX data base is the machine-readable version of the Engineering Index (Monthly/Annual), which provides abstracted information from the world's significant engineering and technological literature. The COMPENDEX data base provides worldwide coverage of approximately 3,500 journals, publications of engineering societies and organizations, papers from the proceedings of conferences, and selected government reports and books.

6. ENVIROLINE, 1971-1984, 102,000 citations, monthly updates.

ENVIROLINE, produced by the Environment Information Center, covers the world's environmental information. Its comprehensive, interdisciplinary approach provides indexing and abstracting coverage of more than 5,000 international primary and secondary source publications reporting on all aspects of the environment. Included are such fields as: management, technology, planning, law, political science, economics, geology, biology, and chemistry as they relate to environmental issues. Literature covered includes periodicals, government documents, industry reports, proceedings of meetings, newspaper articles, films, and monographs. Also included are rulings from the Federal Register and patents from the Official Gazette.

7. ENVIRONMENTAL BIBLIOGRAPHY, 1974-1984, 254,000 records, bimonthly updates.

The ENVIRONMENTAL BIBLIOGRAPHY covers the fields of general human ecology, atmospheric studies, energy, land resources, water resources, and nutrition and health. More than 300 periodicals are indexed in ENVIRONMENTAL BIBLIOGRAPHY, thereby providing quick and easy access to article references for every environment research need.

8. MEDLINE, 1973-1984, 4.1 million records, monthly updates.

MEDLINE (MEDLARS onLINE), produced by the U.S. National Library of Medicine, is one of the major sources for biomedical literature. MEDLINE corresponds to three printed indexes: Index Medicus, Index to Dental Literature, and International Nursing Index. MEDLINE covers virtually every subject in the broad field of biomedicine. MEDLINE indexes articles from over 3,000 international journals published in the United States and 70 countries. Citations to chapters or articles from selected monographs are also included. Over 40% of records added since 1975 contain author abstracts taken directly from the published articles. Over 250,000 records are added per year, of which over 70% are English language.

9. NTIS, 1964-1984, 1,053,000 records, biweekly updates.

The NTIS data base consists of government-sponsored research, development, and engineering plus analyses prepared by federal agencies, their contractors, or grantees. It is the means through which unclassified, publicly available, unlimited distribution reports are made available for sale from such agencies as EPA, NASA, DDC, DOE, HHS (formerly HEW), HUD, DOT, Department of Commerce, and some 240 other units. State and local government agencies are now beginning to contribute their reports to the file.

10. POLLUTION ABSTRACTS, 1970-1984, 102,000 records, bimonthly updates.

POLLUTION ABSTRACTS is a leading resource for references to environmentally related literature on pollution, its sources, and its control. The following subjects are covered by the POLLUTION ABSTRACTS data base: Air Pollution, Environmental Quality, Noise Pollution, Pesticides, Radiation, Solid Wastes, and Water Pollution.

11. WATER RESOURCES ABSTRACTS, 1968-1984, 164,000 records, monthly updates.

WATER RESOURCES ABSTRACTS is prepared from materials collected by over 50 water research centers and institutes in the United States. The file covers a wide range of water resource topics including water resource economics, ground and surface water hydrology, metropolitan water resources planning and management, and water-related aspects of nuclear radiation and safety. The collection is particularly strong in the literature on water planning (demand, economics, cost allocations), water cycle (precipitation, snow, groundwater, lakes, erosion, etc.), and water quality (pollution, waste treatment).

12. WATERNET™, 1971-1984, 11,000 records, quarterly updates.

WATERNET provides a comprehensive index of the publications of the American Water Works Association and the AWWA Research Foundation. Included are books and proceedings, journals, newsletters, standards, manuals, handbooks, and water quality standard test methods. Emphasis is on the technical reports and studies from water utilities, regulatory agencies, and research groups in the United States and its territories, Canada, Mexico, and Latin America. European and Asian data are also reported. The data base is the online counterpart to the index to the Journal AWWA from 1971 to the present, and all AWWA and AWWARF publications from 1973 to the present, with non-AWWA materials included on a selective basis.

Secondary Literature Searches

The secondary literature searches were begun when the hard copies of documents from the primary searches were available for review. All references in the primary documents were considered possible secondary sources.

A significant number of secondary documents were identified on the basis of title alone. Each was retrieved in order to evaluate its value to the study prior to entry into the permanent data base. Thus, documents determined to be of no value to this study were not entered into the permanent data base. Further, each document from this secondary search represented additional possibilities for the identification of additional literature sources.

Author Contact

Authors identified as major contributors to the literature obtained during the primary and secondary searches were contacted. A total of 25 authors were contacted by mail. Most responded, including those located in Europe and South Africa. This effort was relatively fruitful as a number of obscure and unpublished documents of value to the study were obtained.

In addition, personal contacts were made at a number of national meetings. This effort proved fruitful also as additional unpublished data were found. Efforts were made to preview all conference and symposia programs that were considered to be of importance to this study.

Manual Searches

Manual searches of selected current science and engineering journal issues were made. The journals selected for manual searches were those which yielded documents relevant to the study through the primary and secondary literature searches. This activity was maintained throughout the study period.

DOCUMENT RETRIEVAL

Hard copies of documents identified in the literature searches were obtained. Each hard copy document was read by a senior investigator and classified as to its value to the project. Documents were classified into three categories:

1. Reference - a document most likely to be cited in the critical review.
2. Bibliography - a document of some interest but an unlikely source of usable data.
3. Delete from file - a document that could not be placed in either of the first two categories.

Table 6 presents the final status of literature searches.

TABLE 6. CATEGORIZATION OF IDENTIFIED LITERATURE

Category	Number
Reference Documents Reviewed	60
Bibliographic Documents Reviewed	102
Primary References with no Hardcopy	1

MICROCOMPUTER DATA BASE

As each abstract or document was selected for further consideration, it was assigned a serial document number beginning with 0001. The citation was entered into a microcomputer data base developed for this project.

The data base was designed using the pfs:FILE program, a software package of the IBM Personal Computer Professional Series. This program facilitates the creation of a tailored data form for entry and management of a wide spectrum of information (see Table 7). A form was created to accommodate pertinent information on each citation.

TABLE 7. SAMPLE FORM AND INSTRUCTIONS FOR ENTRY INTO THE DATA BASE

INSTRUCTIONS:

INSERT THE INFORMATION AFTER THE COLON ACCORDING TO THE EXAMPLES SHOWN BELOW, DEPENDING ON WHETHER THE REFERENCE IS A JOURNAL ARTICLE, DISSERTATION, GOVERNMENT REPORT, BULLETIN, BOOK, ETC.

BULLETIN.	Dutt, G. R., M. L. Shaffer, and W. J. Moore. 1972. Title. Ariz. Agr. Expt. Sta. Tech. Bull. 101pp.
BOOK.	Author(s) Name(s). Yr. Title. Edition. Publisher. City, State. 37pp.
DISSERTA.	Gupta, S. C. 1972. Title. Ph.D. Dissertation. Utah State University, Logan, Utah. 112pp.
GOV. RPT.	King, L. G. 1973. Title. EPA-R2-73-265, U.S. Environmental Protection Agency. Washington, D.C. 47pp.
JOURNAL.	Nimah, M. N., and R. J. Hanks. 1973. Title. Soil Sci. Soc. Amer. Proc. 37:533-621

FORM:

DATE ENTERED:

:[document serial number]

:[document citation]

YEAR: SOURCE NAME: LAB/FIELD?:
REF/BIBLI?: TRNSP/SURV?: ABST/HRDCOPY?:
INDICATORS: VIRUS: BACTERIA: PARASITE:

Each document was assigned a blank form. Information was entered in the following order:

1. Document's serial document number.
2. Date when information was entered.
3. Document bibliographical information (author[s], year, title, etc.).
4. Year when document was published.
5. Source (Journal, Bulletin, etc.).
6. "Reference" or "Bibliography", depending on its value to the project.
7. "Transport" and/or "Survival", depending on the topic of the article.
8. "Abstract" and/or "Hardcopy", depending on the document on hand at the time.

9. "Laboratory" and/or "field," depending on the type of study.
10. - 13. "Indicator" and/or "Virus" and/or "Bacteria" and/or "Parasites", based on the organism(s) covered by the article.

The program was run on an IBM-XT Personal Computer and permitted arrangement of the documents alphabetically (by first author), or by serial number, date entered, year published, or any other single or combination of items 1-13, above.

It was observed that a number of citations in the computerized data bases used in this study were inaccurate. This necessitated the expenditure of considerable effort in attempting to clarify the intended citations so that the documents could be obtained.

GUIDELINES FOR LITERATURE EVALUATION

During the course of this literature review a broad spectrum of studies ranging from investigations using exogenously added organisms to monitoring of indigenous organisms at field sites was encountered. The challenge posed by such diverse experimental conditions was to qualify the existing data within a common framework reflecting, insofar as possible, expected responses in natural systems. To insure a relatively unbiased appraisal of existing literature, guidelines for the critical evaluation of both laboratory and field studies were described before the review of individual reports began.

Analytical Methods

Perhaps the single most confounding factor in comparing data from various studies is the lack of sensitive, standardized methods for the detection and enumeration of pathogens in sludges and soils. While standard methods for the recovery of selected bacteria, viruses and parasites from potable water are available (American Public Health Association, 1985), little attention has been directed toward optimizing the recovery of pathogens from sludge-amended soils. If, for example, bacterial levels are significantly high, a direct multiple tube inoculation procedure of a sludge-soil suspension may be used. In most instances, however, the level of pathogens present as well as constraints of the assay systems dictate that viruses or parasites be separated from the solid phase in an elution step followed by concentration of the organism prior to assay. As no standardized elution/concentration procedures are widely accepted, numerous methods have been used by different investigators with variable results. For these reasons, strict comparisons of organism recoveries from sludges or soils may not be possible between studies. Within studies, however, the following issues were considered in an attempt to assess the strength of microbiological data:

1. The use of appropriate media and assay conditions;
2. The confirmation/identification of agents;
3. The use of replicate analyses, for development of statements of reproducibility of results;
4. The sensitivity of the methods (i.e., the limits of organism detection); and
5. The quality control procedures used.

Study Design

Good experimental design of either laboratory or field studies is essential to the collection of interpretable data. Attention must be given to when, where, and how samples are collected. Background levels of organisms can be established either by monitoring the test soil prior to sludge application or, preferably, by sampling parallel unsludged soils over the experimental period. Sampling at the time of application is essential to establish the organism load attributable to the introduction of sludge. Thereafter, monitoring schemes should reflect a reasonable frequency of sampling. For example, daily, weekly, or biweekly analysis of experimental plots would allow a timely assessment of organism decay or growth. On the other hand, quarterly or yearly sampling contributes no information to organism behavior, except to document any long-term changes in soil microflora.

Where samples are collected may also influence organism recovery. For example, in a sludge-soil matrix surface samples receiving direct sunlight might be expected to yield fewer viable pathogens than samples collected beneath the surface. In addition, the application of sludge to land results in a very uneven distribution of sludge in soil. To adequately describe the microbial population, multiple samples, collected randomly, should be characterized at each time point. However, sampling logistics and analytical costs could prohibit this approach. Thus, analysis of composite samples representing multiple grab samples presents a viable option.

Finally, the methods used to collect sludged-soil samples or subsurface water beneath sludged fields can greatly influence organism recovery. As an example, equipment used to collect samples should be adequately cleaned or disinfected between sampling locations to avoid cross-contamination. Monitoring subsurface water is also exceedingly difficult. Commonly used ceramic lysimeters can effectively exclude a significant number of both bacteria and viruses depending upon the sorptive characteristics of the soil-water milieu, thus negating the value of analytical measurements. Clearly, choices made by investigators in each of these areas directly influence the interpretability of collective data.

From a regulatory and design standpoint, the collection of supporting data during the course of experimentation or monitoring may provide the most valuable information obtained from a critical review of existing work. Therefore, particular attention was given to the availability of data which might affect pathogen transport and/or survival. Specifically, the collection of site-specific environmental data in the areas of temperature, rainfall, and soil parameters (e.g., composition [sand, loam, clay, etc.], moisture, pH, and organic content) were deemed important.

In addition to the areas discussed above, certain unique aspects of both laboratory-based studies and on-site (field) monitoring studies require special consideration. For purposes of this review, laboratory studies include both bench-scale investigations as well as field studies in which exogenous organisms were added to the sludge prior to land application. The use of high input levels of known pathogens greatly enhances one's ability to recover the seeded organism over time. However, it also may be argued that exogenously added organisms are not incorporated into sludge solids in the same manner as are indigenous organisms and, therefore, they may behave differently with respect to either survival or transport. Furthermore, bacterial cultures grown under optimal nutrient conditions in the laboratory may respond differently to environmental stress when compared to indigenous bacteria selected under nutrient-limiting conditions (i.e., wastewater treatment). Nonetheless, reported laboratory-based studies were used to evaluate the survival and movement of pathogens in sludge-soil systems. In addition to the general points mentioned above, the following factors were considered germane in the comparison of laboratory studies to field studies:

1. The level of organisms added;
2. The documented sludge association of the organisms;
3. The type of sludge used; and
4. The realism of the parameters of the model employed.

Undoubtedly, the most pertinent literature should derive from actual monitoring at operational sludge application sites. The nature of the sludge used dictates both the level and diversity of pathogens entering the sludge-soil matrix. Similarly, the method of sludge application and application rates could affect the pathogen load at a field site during long-term use. For these reasons, the following operational data were noted:

1. Prior sludge treatment;
2. Sludge application rate; and
3. Method of application (e.g., subsurface injection, spraying or surface spreading coupled with tilling).

The issues described above highlight the points considered to be most important in the design of an ideal study addressing pathogen behavior in a sludge-amended soil. These issues represent the authors' cumulative experience based on many years of environmental research as well as a considerable amount of hindsight. Undoubtedly, available literature will fall short in many of these areas; however, the identification of these issues provides a framework within which to evaluate the strength of individual studies.

Organism Inactivation

To provide a common framework from which organism survival could be addressed, simple inactivation values representing 90% (T_{90}) and 99% (T_{99}) dieaway were graphically established. The criteria used in determining these values included:

1. initial monitoring of amended soil within 2 weeks of sludge application;
2. a minimum of three positive, quantitative recoveries over a consecutive monitoring period; and
3. no extrapolation beyond the data reported.

While most of the inactivation values cited in this report reflect initial organism die-away, a few long-term sampling schemes allowed seasonal evaluations of organism survival. In these cases sampling frequencies were generally 1 month or less. One of the most protracted monitoring schemes analyzed using this approach entailed sampling at 1 week, 1 month, 3 months, and 1 year after sludging. In many instances, T_{99} values were not estimated because the experimental data did not reflect a two $\log_{10}$ reduction within the actual sampling period.

It should be emphasized that statistical methods were not used in establishing inactivation rates because, in the opinion of the authors, the strength of the available microbial data did not warrant such an approach. Furthermore, another reader might reach slightly different conclusions in evaluating published reports such that differences between a reported T_{90} of 14 days and 16 days should not be heavily weighed. Finally, one should remember that in microbial systems a persistent population of organisms can exist. Therefore, the dieaway of an organism to levels below the analytical limits of detection may require a longer time than that indicated by the simple application of the T_{90} or T_{99} data. This observation has even greater significance for bacterial pathogens which may have the capability for regrowth under favorable environmental conditions.

SECTION 7

RESULTS

ASSESSMENT OF MICROBIAL METHODS APPLIED TO SOIL SYSTEMS

Before considering the various studies detailing the survival and transport of microorganisms in sludge-amended soils, it is prudent to recognize the limitations influencing quantitative results in such systems. Perhaps the most important problem in evaluating the behavior of microbes in sludges and soils hinges on the methods used for organism recovery. While standard methods for the detection of indicator bacteria in water and wastewater have been widely applied in soil systems, these bacterial groups are not normally associated with human disease. The recovery and enumeration of bacterial pathogens, viruses, and parasites often require elaborate procedures involving a high degree of technical competence and experience. In addition, the factors affecting organism recovery from sludge and soil systems are not well understood. Hence the detection of pathogens at one study site may be different using the same methodology at another site. Furthermore, the recovery of viable bacteria, viruses, and parasites is limited by the volume of sample that can be analyzed, thus imposing in some instances a relatively high sensitivity limit on organism detection.

Salmonella

With few exceptions the studies reporting the recovery of Salmonella from the sludge-soil matrix have used similar approaches. As shown in Table 8, most-probable number (MPN) estimates based on either pre-enrichment or enrichment protocols were used by all investigators. Sludge-soil samples were either inoculated directly into the specified media or first mixed with buffer to create a homogenous, pipetable suspension for analysis. In virtually all cases, the eventual identification of Salmonella isolates culminated with serological identification. Based on these procedures, the isolation and quantitation of Salmonella species (sp.) should be highly specific, i.e., few false positives. However, it should also be understood that since the isolation medium used was different in many cases, the sensitivity of Salmonella detection could likewise be variable. One study, cited by Jones and associates (1983), illustrates this point. When grass samples from sludged pastureland were analyzed for Salmonella by two different regional water laboratories using different media, dramatic differences in organism levels were observed. One laboratory consistently reported bacterial levels 10- to 100-fold higher than the other laboratory. Not surprisingly, Salmonellae were recovered through the entire 10 week study period by the first laboratory, and only through 9 weeks by the second. Nonetheless, inactivation rates were similar.

TABLE 8. MPN METHODS USED FOR THE ISOLATION OF SALMONELLAE FROM SLUDGE-SOIL SYSTEMS

Reference	Pre-Enrichment	Enrichment	Plating	Confirmation	
				Biochemical	Serological
Watson, 1980	PBP, 37C, 18-24 hr	MKT, 42C, 24 & 48 hr	BGA & DCA, 37C	API20E®	Polyvalent O&H
Larkin, et al., 1978	None	DSE, 40C, 24-48 hr	XLD agar, 37C	KIA or TSI	Yes-Unspecified
Strauch, et al., 1981	PW, 37C, 18-24 hr	MKT, 45C-15 min. 37C-24 hr	BGA & BTBA,	Yes-Unspecified	Yes-Unspecified
Andrews, et al., 1983	None	TTRB+Cefsulodin	BSA	None	Yes-Unspecified
Berron, et al., 1981	None	Selenite Broth, 42C, 24 hr→ Hektoen Broth, 37C, 24 hr	None	None	None
Watkins & Sleath, 1981	PBP, 25C, 18-24 hr	RB, 41C, 24 & 48 hr	XLD agar, 37C	None	Polyvalent O&H
Yanko, et al., 1976,1978	None	TB, 37C, 18-24 hr	BSA & XLD, 37C	TSI	Polyvalent O
Jones, et al., 1983	PBP, 37C,	MKT or RB, 42C, 18-24 hr	BGA or XLD, 37C	Not Specified	Not Specified
Dickson & Tribe, 1986	PBP, 37C, 18-24 hr	RB, 40C, 48 hr	XLD agar, 37C	KIA and urea agar	None

PBP = phosphate buffered peptone
MKT = Muller Kauffmann tetrathionate broth
BGA = Brilliant green agar
DCA = Deoxycholate citrate agar
KIA = Kligler iron agar
PW = peptone water
BTBA = brom thymol blue agar

TTRB = tetrathionate broth (Rolfe's)
BSA = bismuth sulfite agar
DSE = dulcitol selenite broth
XLD = xylose lysine deoxycholate
TB = tetrathionate broth
TSI = triple sugar iron
RB = Rappaport broth

Other Bacteria

Individual papers included in this review have addressed the survival of several unique bacterial species of some public health significance, which, on the basis of microbial characteristics, could survive well in the water and soil environment. Specifically, representative Mycobacterium (Van Donsel and Larkin, 1977), Clostridium (St. John and Matches, 1982), and Listeria (Watkins and Sleath, 1981) species have been recovered from sludge-soil samples. While exogenously added Mycobacterium bovis was enumerated using an agar-based pour plate technique, both indigenous Clostridium perfringens and Listeria monocytogenes were isolated using MPN procedures. In all instances, however, whole sludge-soil samples (i.e., 10% soil suspensions) were analyzed.

Mycobacterium bovis was verified by the growth rate and morphology of colonies on defined medium and by characteristic acid-fast (Ziehl-Neelsen) morphology of bacterial cells. Listeria sp. were identified based on characteristic colony coloration and serological testing. Clostridium sp. were scored on the basis of typical stormy fermentation in an iron-milk medium, without further testing. Since certain species of mycobacteria and clostridia are ubiquitous in soil, levels found in sludge-amended samples were compared when possible to unsludged soils in evaluating the behavior of these organisms.

Indicator Bacteria

In virtually all of the studies evaluated, a most probable number (MPN) procedure as described for total coliforms, fecal coliforms, and fecal streptococci in wastewater (American Public Health Association, 1985, or earlier edition) was applied to the enumeration of these bacterial groups in sludge-soil samples. Test samples were mixed with either peptone- or phosphate-buffered water to achieve a relatively homogenous suspension (generally 10% soil) before being inoculated into selective media. Presumably, total coliforms were subjected to both a presumptive and a confirmed test. In no case was it clearly stated that a completed coliform test series was done. Since a completed test involves both plating of the confirmed sample onto either Endo or EMB agars and subsequent Gram staining of typical coliform colonies, it is unlikely that such extensive analyses were carried out routinely. As defined in Standard Methods, an MPN test for fecal coliforms requires only a transfer from the positive presumptive tubes of the total coliform test (lactose or lauryl tryptose broth) into EC medium which is incubated at the elevated temperature of 44.5°C. Gas production within 24 hours is recorded as evidence of fecal coliform growth. Fecal streptococci are enumerated by growth at 35°C in a dextrose azide broth followed by confirmation in ethyl violet azide broth.

Undoubtedly an MPN procedure is the most desirable standard assay for bacterial detection, assuming a homogenous sample can be achieved. Although used in selected studies, membrane filtration procedures are largely incompatible with direct analysis of soil suspensions. Miller (1974), for example, noted the interference of clay and silt in the development of bacterial colonies on the membrane surface. Furthermore, the recovery of viable bacteria from sludges and soils can be very irreproducible, thus negating the enumeration of organisms by filtration of soil eluates. Using parallel enumeration of total and fecal coliforms, Sekla and Stackiw (1983) repeatedly failed to recover indicator bacteria from sludge-amended soils using membrane filtration procedures although MPN values were positive up to one year post-injection. MPN procedures are limited, however, by the volume or weight of sample that can be analyzed. For example, from a 10% sludge-soil suspension (10 g/90 ml diluent), a single positive reaction in a 5-tube, 10 ml-1 ml-0.1 ml series would result in an MPN index of 0.2 organisms/g.

It must be remembered that the tests defining indicator organisms were developed to define fecal pollution of the water environment. Application of these analyses to soil systems is confounded by the fact that a variety of indigenous soil microorganisms can give positive reactions in these indicator tests. Therefore, tests for total and fecal coliforms and fecal streptococci were interpreted against the background levels of these bacterial groups in parallel control plots when such data were available.

Human Viruses

In the absence of standardized procedures, several methods have been applied to the recovery of viruses from sludge-soil systems. Virtually all reported techniques covered as part of this review used an alkaline pH buffer to release bound viruses. After removing the solids, most often by centrifugation, viruses can be concentrated from this primary eluate by a number of procedures. In the most widely used approach, the pH of the eluate was lowered to 3.5-4.5 to facilitate optimal flocculation of proteins. The resulting viral-containing floc was collected by centrifugation and viruses were finally released in a small volume of phosphate- or serum-based buffer. An alternate viral concentration procedure employed dialysis of the primary eluate against polyethylene glycol, resulting in a sample volume reduction by dewatering. In laboratory studies in which viruses such as polio- and echo-virus were added to sludge-soil systems, reported recovery efficiencies have ranged from a low of 8% (Metropolitan Sanitary District of Greater Chicago and IIT Research Institute, 1979) to maximal values of 70%-80% (Hurst et al, 1978; Metropolitan Sanitary District of Greater Chicago and IIT Research Institute, 1979).

The detection of human viruses in environmental samples is also governed by the assay system used. Human enteric viruses, represented by polio-, Coxsackie- and echo-viruses, are routinely grown on primate cell lines. However, a number of researchers have reported that the frequency of a specific viral isolation is dependent on the cell lines employed. An extensive study comparing the sensitivity of five primate cell lines for enterovirus recovery was reported by Schmidt and co-workers (1978). A total of 181 different wastewater samples were assayed on primary Rhesus monkey kidney (RhMK), Buffalo Green monkey (BGM), monkey fetal kidney (MFK), human embryonal rhabdomyosarcoma (RD), and human fetal diploid kidney (HFDK) cells in both tube and plaque assay systems. Their results showed that from environmental samples, polioviruses were recovered most often on RD and BGM cells; Coxsackievirus Group B, on BGM; echoviruses, on RD and RhMK; and reoviruses on RhMK and MFK. Group A Coxsackieviruses were recovered on HFDK and RD cells only. Work reported by Morris and Waite (1980) favored the use of BGM and RD cells over Vero cells in an agar cell suspension assay.

Similarly, Moore and associates (1981) observed generally higher viral recoveries with cell suspension assays. Of the cell lines tested, HeLa, BGM, and RD cells consistently yielded the greatest number of viruses from wastewater samples by plaque assay methods. Broad-spectrum viral isolations including polio-, Coxsackie-A and B, and echo-viruses were obtained with HeLa cells, while RD cells exhibited a more weighted recovery of echoviruses. Hamparian and associates (1985) likewise observed that RD cells were most useful for the isolation of echoviruses from digested sludges, while most Coxsackievirus B recoveries were made from BGM and HeLa cell lines.

Unfortunately, field studies included as part of this review have relied almost exclusively on viral recovery using a single cell line, most frequently BGM cells. Furthermore, several viruses of great public health significance are more difficult to grow in vitro (e.g., hepatitis A virus and rotaviruses) or have not yet been successfully cultivated outside of a human host (e.g., Norwalk and related viruses and non-A, non-B hepatitis virus). Thus, no description of the behavior of these viruses in sludge or soil systems is currently available.

Parasites

Among the various microbial groups, the detection of parasites in environmental samples is undoubtedly the most difficult. Protozoan cysts and helminthic ova are fecally excreted by infected hosts. Their enumeration in wastewater or sludge is impeded by the facts that parasites in these stages of development do not multiply in culture and that their numbers are generally too low to allow direct detection. Thus, as with viruses, concentration techniques are required. Subsequently, samples must be microscopically examined by a highly trained technician to identify and enumerate individual ova or cysts on the basis of known morphology. Parasite cysts and eggs must also be differentiated from those of free-living protozoa, nematodes, and other invertebrates.

In sludges and sludge-amended soils the major emphasis has been placed upon the detection of helminthic ova. Because of their size, eggs of nematodes such as Ascaris are more likely to be found in primary sludges. Furthermore, Ascaris ova are more resistant than cysts to sludge digestion processes (Hays, 1977). These facts coupled with the resistance of Ascaris eggs to environmental stresses such as desiccation have resulted in the use of this nematode as a benchmark for parasite destruction.

In the studies evaluated as part of this review, two general approaches have been used to separate parasitic ova from sludge-soil samples. One procedure involved mixing the sludged soil with detergent and water to release eggs or cysts and subsequently removing large particulates by low speed centrifugation (Lehmann et al, 1983). The resulting supernatant was then concentrated using techniques reviewed below. Alternatively, soil samples were wet sieved to remove particulates larger and/or smaller than parasitic eggs or cysts (Theis et al, 1978; Graham, 1981). These parasitic stages were then recovered from the remaining soil fractions using one of several concentration techniques.

Conventional concentration techniques are based on density differences between parasitic ova and cysts when compared to other particulate matter present in a given sample. Specifically, flotation procedures using sucrose or a variety of salts including sodium nitrate and zinc sulfate were used to recover eggs and cysts in studies included in this review (Theis et al, 1978; Graham, 1981; Lehmann et al, 1983; Carroll and Ross, 1983). Alternatively, sedimentation of ova and cysts through formalin or formalin-ether mixtures was also recorded as a concentration method (Theis et al, 1978; Carroll and Ross, 1983).

The diverse combination of detection methods as well as the subjective nature of the final microscopic analysis highlight the problems encountered in the interpretation of parasite data in sludge-soil systems. Generally, concentration efficiencies of 50% to 80% are cited for the recovery of parasitic ova and cysts from sludges (Little, 1985). Using seeded sludge-soil samples, recovery efficiencies ranging from 80% (Graham, 1981) to 8% (Lehmann et al, 1983) were noted. Furthermore, Lehmann and associates (1983) noted a rapid loss of organisms (within 20 minutes) during zinc sulfate flotation.

The relatively small volume of sample that ultimately can be examined further compounds the problem of parasite detection. Flotation and sedimentation techniques are usually limited to a sample volume of less than 10 ml. Even in those instances where 2 kg of soil were wet-sieved, only 25 ml of the resulting sample were concentrated (Theis et al, 1978). Therefore, positive results should be evaluated within the context of each individual study and negative results should be viewed with consideration for the limitations of detection sensitivity.

SURVIVAL OF MICROORGANISMS IN SLUDGE-AMENDED SOILS

While a major goal of this literature review was to compile and critically evaluate available work describing the survival of pathogenic microorganisms in sludge-amended soil systems, it soon became evident that very little published work dealing with organisms of public health significance was available. Among bacteria, only Salmonella sp. have been widely studied. Limited data were found dealing with other bacteria such as Clostridium sp. and Mycobacterium sp. By far, most investigations have focused on indicator bacteria including total coliforms, fecal coliforms and fecal streptococci. Likewise, few studies have successfully described the fate of viruses or parasites in sludges applied to land.

Because of the paucity of scientific reports, essentially all literature retrieved as part of this study describing the survival of microorganisms in sludge-amended soil has been considered. The strict application of guidelines for the evaluation of these studies as described in the previous section (METHODS) was not possible as virtually no published report provided adequate information covering all aspects of methodology and study design. Particularly lacking were quality assurance data and real-time environmental measurements such as temperature, soil moisture, and rainfall. However, an effort has been made to highlight deficiencies within a study when it was felt that data qualification was necessary due to constraints in methodology or study design.

To facilitate the comparison of available studies, each major microbial group is reviewed. Both laboratory and field studies are described and discussed.

Salmonella

While the level of Salmonellae in domestic wastewater may be relatively high compared to other bacterial pathogens (see Table 2), their detection in sludge and soil environments is often difficult. Consequently, while many field studies have been undertaken with great expectations, frequently the final compilation of data results in a qualitative assessment of Salmonella persistence (See Table 9, Part A).

In an early report, Köser (1967) described long-term monitoring of sludged pastures in the Federal Republic of Germany. Beginning in March, 2 days after sludge application to a depth of 20 to 30 mm, grass, topsoil to a depth of 1 cm, and an overlaying sludge crust were assayed for Salmonella. Both the total number of samples analyzed and the number of positive samples were reported. At the end of 4 and 5 weeks, 12% of grass samples were still yielding Salmonella; however, no positive recoveries were made after the fifth week. In the same fields, topsoil yielded Salmonella through 10 weeks with well over 50% of all samples consistently being positive through 5 weeks. In parallel studies, 1 to 2 cm of dried sludge crust were analyzed. Salmonellae were recovered from these samples throughout the entire 16 week monitoring period.

Similar observations were made at a field site in southeastern Pennsylvania where aerobically digested sludge was applied to the soil surface in April (Kenner et al, 1971; Cooke, 1969). Salmonellae were recovered from soil samples (0 to 2 cm depth) for 10 weeks.

Over a 2 year period, Yanko and associates (1978) observed the levels of indigenous Salmonella in sludge-amended soils as part of a study conducted with the University of California, Riverside. Both liquid digested and composted sludges were disced into fields at loading rates as high as 90 dry metric tons per hectare (t/ha). Salmonellae were recovered from fields 4 and 5 months after sludge application (Table 9); however, they were also recovered from one control field at the beginning of the winter test series. Subsequently, in a special small scale study in which more frequent sampling was completed, Yanko and associates (1978) reported the recovery of Salmonella for as long as 16 to 20 weeks. In sludged soil planted with a cover crop and maintained at a 0.2 to 0.8 bar moisture tension, 150 MPN/g was recovered at the end of the study while 10 MPN/g was observed in the dry soil. Once again sporadic and low Salmonella levels were detected in unsludged control soils.

TABLE 9. LONGEVITY OF SALMONELLA IN SLUDGE APPLIED TO LAND*

Reference	Sludge Type	Application	Season	Sample Type	Last Positive Sample, Post Application
Part A. Indigenous Organisms					
Köser, 1967	Raw	Surface spread	Spring-Summer	Grass Soil Sludge crust	5 wks 10 wks 16 wks
Kenner, et al, 1971	Digested (aerobic)	Surface spread +	Spring-Summer +	Soil	10 wks
Yanko, et al, 1978	Digested (anaerobic)	Spread, disced	Winter	Soil	5 mo
	Digested (anaerobic)	Spread, disced	Summer	Soil	4 mo
Berron, et al, 1981	Thickened	Surface spread	Winter	Soil Sludge crust	15 days 1 mo
Carroll and Ross, 1983; Sekla and Stackiw, 1983	Raw	Subsurface injected	Summer	Soil	3 mo
Part B. Exogenous Organisms					
Hess and Breer, 1976	Raw #	Surface spread	Winter	Grass Topsoil	61 wks 57 wks
Strauch et al, 1981	Digested ** (anaerobic)	Surface spread	Summer Winter	Sludge Sludge	424-820 days 104-350 days

* Does not include studies compiled in Table 10.

+ Cooke, 1969.

# *S. typhimurium* added to 10^6 organisms/L of applied sludge.

** *S. seftenberg* added to 10^7 organisms/ml of sludge.

French researchers were able to recover *Salmonellae* from a thickened domestic sludge spread onto a fallow field during the winter season (Berron, et al, 1981). Although the levels of these organisms were near the limit of detection, positive recoveries after sludge application were made from soil at 15 days and from the overlaying sludge crust at 1 month.

The City of Winnipeg, Manitoba, Canada, included monitoring for *Salmonella* sp. in a study directed at assessing the feasibility of injecting municipal sludge into agricultural fields (Carroll and Ross, 1983; Sekla and Stackiw, 1983). Both raw and anaerobically digested sewage sludges were injected at a depth of 15 cm. In-plant monitoring showed that *Salmonella* could be recovered from 50 to 83% of the raw sludges, but only 17% of the digested sludge samples yielded this pathogen. In one plot receiving untreated sludge, *Salmonellae* were recovered from within the injection trench 1 week (50% of samples positive at 15 cm depth), 3 weeks (67% positive at 15 cm), and 3 months (33% positive at 7.5 cm) after sludge application. Final samples taken 1 year post injection were negative. In a second plot injected with raw sludge, positive *Salmonella* recoveries were made at 1 week and 1 month. No viable *Salmonellae* were detected at any time in soils amended with anaerobically digested sludge.

Taken together, these five studies based on the recovery of indigenous bacteria suggest that *Salmonella* can persist in the sludge-soil environment for a period of 4 to 5 months. From the standpoint of site management, however, it is perhaps more important to define the rate of organism inactivation. With this in mind, the time required to achieve 90% and 99% *Salmonella* dieaway was compiled from studies in which sufficient quantitative data were available (see Table 10). Additional information describing the sludge, its application, and study site characteristics were also recorded.

In 1971 Kenner and associates reported data from field studies in which both digested and raw activated sludges were spread on test plots located in Cincinnati, Ohio. Soil samples were devoid of *Salmonella* prior to sludge addition. Sludge was applied in November and monitoring continued until June of the following year. The plot receiving digested sludge yielded only sporadic *Salmonella* isolates. However, positive *Salmonella* recoveries were made from the top 5 cm of sludge-treated soil through 66 days on one plot and 87 days on a second plot receiving raw sludge. During this winter period temperatures were unusually cold for the region, ranging from a low of -19°C to a high of 18°C. Total precipitation of 47 cm and 54 cm was recorded through 66 days and 87 days, respectively. Under these conditions, T_{90} values of 15 days and 12 days were observed (Table 10, line codes Q and R). In one plot where sufficiently high levels of *Salmonella* were present initially, a two $\log_{10}$ reduction (T_{99}) required 22 days. Interestingly, a wide spectrum of *Salmonella* serotypes was recovered from the soil samples. In one plot, six of nine serotypes found in the applied sludge were recovered from the soil while in a second test plot, only 13 of 23 sludge-associated serotypes were isolated from soil samples.

Studies conducted during a summer season in the United Kingdom observed *Salmonella* survival to be from 6 to 7 weeks in tilled soil (Watson, 1980). Anaerobically digested sludges were applied to sandy or organic soils and

TABLE 10. SUMMARY OF SALMONELLA SURVIVAL IN SLUDGE APPLIED TO SOIL

PART I.

Line Code	Reference	Sludge Type	% Solids	Sludge Application	
				Rate (t/ha)	Mode
A	Watson, 1980	Digested (Anaerobic)	3.6	255	Sprayed, tilled
B	Watson, 1980	Digested (Anaerobic)	5.5	391	Sprayed, tilled
C	Watson, 1980	Digested (Anaerobic)	1.1	77	Sprayed, tilled
D *	Larkin, et al, 1978	N.I.	--	--	N.I.
E *	Larkin, et al, 1978	N.I.	--	--	N.I.
F	Andrews, et al, 1983	Raw +	4.3	5	Injected
G	Andrews, et al, 1983	Raw +	4.3	5	Injected
H	Watkins and Sleath, 1981	N.I.	--	--	Sprayed

PART II.

Line Code	Soil Characteristics			Mean Ambient Temp. (°C)	Initial Microbe Conc. **	Die-off			
						Surface (0-5 cm)		Depth (5-15 cm)	
	Type	Moisture (%)	pH			T_{90}(d)	T_{99}(d)	T_{90}(d)	T_{99}(d)
A	Sand	14	4.8	9	0.16 (a)	--	--	16	33
B	Sand	13	5.9	9	0.035 (a)	--	--	14	29
C	Rich Loam	--	--	--	--	--	--	22	45
D *	N.I.	--	--	--	1E8	10	18	--	--
E *	N.I.	--	--	--	1E8	6	11	--	--
F	Light Sand	--	8.0	15 #	0.7-2.1 (b)	--	--	4 ++	7 ++
G	Light Sand	--	8.0	9 #	0.3 (b)	--	--	17 ++	30 ++
H	N.I.	--	--	--	1.3 (b)	11	24	--	--

N.I. = No Information
* Organisms Added
+ Significant Industrial Component
# Soil Temperature
++ 25-30 cm

** Units Reported: (a) MPN/g sludged soil (wet wt)
(b) MPN/g sludged soil

TABLE 10. SUMMARY OF SALMONELLA SURVIVAL IN SLUDGE APPLIED TO SOIL (Continued)

PART I.

Line Code	Reference	Sludge Type	% Solids	Sludge Application	
				Rate (T/Ha)	Mode
I	Jones, et al, 1983	N.I.	--	--	Spread
J	Jones, et al, 1983	N.I.	--	--	Spread
K	Jones, et al, 1983	Raw	--	--	Spread
L	Jones, et al, 1983	Raw	--	--	Spread
M	Jones, et al, 1983	Digested	--	--	Spread
N	Jones, et al, 1983	N.I.	--	--	Injected
O	Jones, et al, 1983	N.I.	--	--	Injected
P *	Bergstrom and Langeland, 1981	Raw	20	213	Mixed
Q	Kenner et al, 1971	Raw, Activated	--	--	Spread
R	Kenner et al, 1971	Raw, Activated	--	--	Spread
S	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
T	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
U	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
V	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried

PART II.

Line Code	Soil Characteristics			Mean Ambient Temp. (°C)	Initial Microbe Conc. **	Die-off			
						Surface (0-5 cm)		Depth (5-15 cm)	
	Type	Moisture (%)	pH			T_{90}(d)	T_{99}(d)	T_{90}(d)	T_{99}(d)
I	N.I.	--	--	15	1.2E7 (a)	23	39	--	--
J	N.I.	--	--	4	1,600 (a)	61	--	--	--
K	N.I.	--	--	--	120 (c)	25	45	--	--
L	N.I.	--	--	--	--	9	19	--	--
M	N.I.	--	--	--	250,000 (b)	11	21	--	--
N	N.I.	--	--	15	2.1 (b)	--	--	6	--
O	N.I.	--	--	14	1.0 (b)	--	--	22	--
P *	N.I.	--	--	--	7,000 (b)	--	--	14 #	28 #
Q	N.I.	--	--	-1	0.5 (b)	15	--	--	--
R	N.I.	--	--	1	2.3 (b)	12	22	--	--
S	Heavy Clay	--	--	12 ++	220 (d)	--	--	11	22
T	Medium Clay Loam	--	--	12 ++	220 (d)	--	--	8	18
U	Light Sand	--	--	12 ++	220 (d)	--	--	11	22
V	Light Sand	--	--	15 ++	220 (d)	--	--	3	7

N.I. = No Information
+ Significant Industrial Component
* Organism Added
++ Soil Temperature
# Sampled to Depth of 25 cm

** Units Reported:
(a) MPN/100 ml diluent (on grass)
(b) MPN/g sludged soil
(c) MPN/g (on grass)
(d) MPN/100 ml sludge soil mixture

tilled for planting after 2 weeks. Longer Salmonella survival as indicated by a T_{90} of 22 days and a T_{99} of 45 days was observed in the heavier organic soil, which also received the lowest sludge loading (see Table 10, line code C). Bacterial dieaway was more rapid in the sandy soil with an average T_{90} of 15 days and a T_{99} value of about 30 days (Table 10, line codes A and B). Even though the sludge loadings were significantly different between the fields, the desiccation that occurred in the sandy soils may have contributed to the more rapid inactivation of Salmonella.

Andrews and coworkers (1983) sought to determine whether Salmonella sp. survived for longer periods if sludge was injected beneath the soil surface. By using sludge from a treatment plant receiving significant quantities of poultry processing waste, higher levels of indigenous Salmonella were anticipated. Raw sludge was injected into a light sandy soil at a depth of approximately 25 to 30 cm. After injection in July, Salmonellae were isolated from the injection trench for only 1 week with a T_{90} of about 4 days (Table 10, line code F). Following sludge application in February, Salmonellae were consistently recovered through 24 days with intermittent isolations until 66 days. The T_{90} for this winter period was 17 days (Table 10, line code G). During the two study periods precipitation amounts were roughly equivalent. The longer survival may therefore be correlated with a lower soil temperature during the winter.

Likewise, Dickson and Tribe (1986), working at the University of Cambridge, England, used raw sludge from a treatment plan receiving poultry processing waste to evaluate Salmonella survival in three soil types under controlled laboratory conditions. To simulate sludge injection, glass tubes packed with soil were loaded with sludge and buried at a 20 to 35 cm depth in temperature-equilibrated bins filled with the homologous soil type. Control tubes received an equivalent amount of water prior to burial. Grass turf covered each bin and an equivalent of 5 cm of water per month was applied to this surface each week. Bins were exposed to 12 hour daylight/darkness cycles. At weekly intervals, control and test samples were retrieved and the entire content of each tube was analyzed for selected bacterial groups including Salmonella. Bacterial survival was evaluated over a relatively narrow range of temperatures (9°C, 12°C, and 15°C) for an 8 week period. Dickson and Tribe (1986) concluded that the rate of organism decline was unaffected by soil type or temperature. Further, using linear regression analysis based on first order decay kinetics, a T_{90} for Salmonella of 1.3 weeks was reported. Graphical interpretation of the experimental data within the guidelines established for this review led to similar interpretations with one exception. Salmonella inactivation was comparable in both heavy clay and clay loam soils at all three temperatures and in sandy soil at 9°C and 12°C. Representative T_{90} values for the constant 12°C tests as shown in Table 10 (line codes S, T, and U) indicate 10% survival at 8 and 11 days. However, a more rapid dieaway of Salmonella could be plotted from experimental data collected for the sandy soil at 15°C with an approximate T_{90} of 3 days (Table 10, line code V). The combination of higher temperatures and a more readily desiccated soil could be cited as factors favoring a more rapid bacterial inactivation in this case.

Studies with more limited descriptive information include work reported by Watkins and Sleath (1981). These workers isolated Salmonella for 5 weeks following spray application of sludge to a field. Interpretation of their graphical data showed a T_{90} of 11 days and a T_{99} of 24 days (Table 10, line code H). Similar Salmonella dieaway was reported by Godfree and associates (described by Jones, et al, 1983) who observed virtually identical losses of Salmonella in both raw and digested sludges applied to land with T_{90} values of 9 days and 11 days, respectively (Table 10, line codes L and M).

Results of several studies completed under the auspices of the North West Water Authority in the United Kingdom have been summarized by Jones and associates (1983). Three separate monitoring efforts evaluating the survival of Salmonella on grass growing on sludge-sprayed pastureland resulted in T_{90} values of 23 days for studies completed in September to October (mean temperature, 15°C); 25 days in October to January (no temperature cited); and 61 days in January to February (mean temperature, 4°C) (Table 10, line codes I, K and J respectively). A two $\log_{10}$ reduction of indigenous Salmonella required 39 days and 45 days for the first two studies cited. In the absence of other supportive data, colder temperatures favored a longer survival time in these cases. However, in evaluating the efficacy of subsurface sludge injection, Matthews and his colleagues (cited by Jones, et al, 1983) observed 90% loss of indigenous Salmonella in about 6 days for late summer injection (15°C) and in 22 days for late winter injection (14°C) (Table 10, line codes N and O) in spite of very similar temperature averages during the two seasons. Hence other factors such as unique attributes of a given sludge or metabolic activities of soil microflora may also play a significant role in bacterial dieaway.

With one exception associated with low ambient temperature (Jones et al, 1983), researchers monitoring the fate of indigenous bacteria have reported a range of T_{90} for Salmonella on or close to the soil surface of 9 to 25 days. Only 1% of the naturally occurring Salmonella sp. could be recovered near the surface within 50 days after sludge application with T_{99} values ranging from about 20 to 45 days. Limited data at deeper depths seem to suggest similar survival times when sludge was tilled into the soil (Watson, 1980). Likewise, Salmonella in sludge injected to a depth of 30 cm exhibited analogous dieaway except in a single case where a 99% reduction was observed in 7 days with a recorded soil temperature of 15°C (Andrews et al, 1983).

In spite of the relative consistency of these data, questions may be raised regarding the strength of these results based, in general, on low numbers of Salmonella in the sludge-amended soil. Specifically, when initial levels of ≤ 2 MPN/g serve as the baseline against which inactivation is assessed, the sensitivity and reproducibility of analytical methodology result in essentially Salmonella presence or absence data. To enhance the detection sensitivity of Salmonella introduced into the soil environment, therefore, several researchers have added laboratory grown bacteria to sludge prior to its application to land.

In laboratory experiments, Hess and Breer (1975) followed the survival of Salmonella on sludge-contaminated grass under conditions of controlled relative humidity and temperature. In one completed series of experiments, *Salmonella* originally seeded at 10^7 organisms/ml was detected for 15 weeks at 4°C and 29 weeks at 18°C when the relative humidity exceeded 90%. At low humidity (<30%) *Salmonella* persisted for 72 weeks at 4°C and 41 weeks at 18°C. In a subsequent paper, Hess and Breer (1976) reported results of a duplicate study in which 4 x 10^7 *Salmonella*/ml were applied to grass in sludge. At a humidity in excess of 90%, *Salmonellae* were recovered for 17 weeks at 4°C and 42 weeks at 18°C. Once again at low humidity (<30%) this bacterium showed extended survival with recoveries at 104 weeks at both 4°C and 18°C. Apparently, excessive moisture was detrimental to the bacteria. However, due to the lack of quantitative data (i.e., number of organisms recovered at any given time), the actual relationships between organism decay and/or regrowth cannot be ascertained. For example, if rapid organism growth occurred, the initial depletion of nutrients could have accounted for shortened overall survival times. In a subsequent field study, the same researchers added 10^6 *S. typhimurium* to each liter of raw sludge, which then was used to fertilize a grass bed. Under "normal" winter conditions, *Salmonella* was recovered for a period of 61 weeks on grass and 57 weeks in the overlaying humus layer (see Table 9, Part B).

As part of a long-term study on *Salmonella* persistence in sludge applied to land, Strauch and associates (1981) added *S. seftenberg* to anaerobically digested sludge at a concentration of 10^7 organisms per ml. The sludge was then spread on experimental plots within eleven different forest stands located in the Federal Republic of Germany. Various soils representing sand, loam, and clay bases were included in the study. Their pH values (in water) varied from pH 3.6 to 3.9 for sand to pH 4.8 to 5.0 for clay. Beginning in June (summer) approximately 100 m^3 of sludge were applied per hectare. Sludge was applied to a second series of plots in December (winter) using twice the summer loading (200 m^3/ha). Monthly sampling was then conducted. The mean yearly temperature was reported as 8.3°C while average yearly precipitation in the region of the study was 74 cm. Results from this long-term study were presented as days of organism viability, once again allowing only a qualitative approach for data interpretation. Sludge controls held in the laboratory at 4°C contained recoverable *Salmonella* through 270 days for the summer trial and 258 days for the winter trial. At sites receiving seeded sludge in June, *Salmonella* persisted in the sludge layer as long as 820 days on one plot. In two fields the pathogen was recovered for 424 and 452 days; on four plots, for 516 to 580 days; and on four plots, for 640 days. The results were dramatically different when *Salmonella* contaminated sludge was applied in the winter. Under these conditions the longest survival time recorded was 350 days with most *Salmonella* persisting for less than 250 days (eight plots, 104 to 230 days). On two experimental fields, organisms were recovered from the sludge layer through 322 days. No clear association between soil type and survival was evident, although heavily wooded forest stands appeared to be associated with longer survival. Presumably these plots were subject to less solar influence, i.e., reduced heat and ultraviolet irradiation. A possible explanation advanced by the authors for extended *Salmonella* persistence following summer application was that organism growth and/or acclimation occurred prior to the onset of less favorable winter conditions. Surface temperatures as high as 43°C, conceivably favorable to *Salmonella* replication, were measured during the summer months.

Quantitative data using S. typhimurium seeded into sludge in Cincinnati, Ohio, have been reported by Larkin and co-workers (1978). Vegetable plots planted with either lettuce or radishes were sprayed with liquid sludge containing in excess of 10^7 organisms per ml. Soil samples were taken immediately following application and subsequently at weekly intervals. In an experimental series initiated during the 1975 growing season, Salmonella in the sludge-soil matrix (0 to 4 cm depth) decreased linearly through 6 weeks, but was sporadically detected to week 12. A T_{90} value of about 6 days and a T_{99} of 11 days could be ascertained from the 1975 data (Table 10, line code E). During the 1976 study, Salmonella dieoff was somewhat slower with T_{90} and T_{99} values of approximately 10 and 18 days, respectively (Table 10, line code D).

More recently, Bergstrom and Langeland (1981) conducted a series of experiments at the Norwegian Higher School of Agriculture in Oslo. Small plots (100 cm W x 80 cm L x 25 cm D) were filled with a mixture of 200 kg of humus and 17 kg of sewage sludge (approximately 20% solids). S. typhimurium (about 2 x 10^{10} organisms) was added to the sludge-soil mixture. Vegetable crops including radishes, turnips, and lettuce were planted in seven of the ten sludge-treated plots. Soil samples to a depth of 25 cm were analyzed for Salmonella at the beginning of the study and at 7 and 10 weeks. Initial analysis showed a concentration of about 7×10^5 Salmonella per 100 g of soil that was reduced by a factor of about 10^4 in 10 weeks. A T_{90} value of 14 days and a T_{99} value of about 28 days could be estimated from available data (Table 10, line code P). Interestingly, the initial soil analysis accounted for less than 10% of the Salmonella theoretically used to seed the experimental plot. Thus, the recovery of S. typhimurium, in this case by inoculation into selenite broth, appears to have been very low.

Even though these last two studies were completed under different environmental and experimental conditions, the T_{90} values for S. typhimurium were relatively close to one another: 6 to 10 days in the Ohio study and 14 days in the Norway study. Furthermore, both the T_{90} and T_{99} values were well within the range of indigenous Salmonella inactivation cited above.

Still another aspect of Salmonella persistence in sludges applied to agricultural lands, namely the dissemination of this pathogen into cattle herds grazing on sludge-amended pastures or on fodder from sludged fields, has received widespread attention in Europe. In monitoring pastures sprayed with anaerobically digested sludge in the Netherlands, Kampelmacher and van Noorle Jansen (1974) also collected feces from cattle pastured on fields 1 to 3 weeks after sludge application. Fecal samples were collected at random either from the pastures or from barn areas. Animals were pastured on sludged fields between 8 to 21 days after application for a period of about 1 week. Of 250 fecal samples, 11 yielded various Salmonella biotypes, all of which were also recovered from sludge being sprayed. The authors noted that this isolation rate of 4.4% was significantly higher than previous studies of healthy cattle in which Salmonella were recovered from 0.3% to 0.6% of the animals tested at slaughterhouses. It should be noted, however, that individual animals were not monitored due to the experimental design of this study, as specimens were collected from common grazing or stabling areas. Furthermore, actual monitoring of parallel herds not exposed to sludged fields would be a preferable control rather than the use of historical infection and/or carrier rates as measured by the isolation of Salmonella from the organs of slaughtered cattle. Nonetheless, the higher frequency of positive fecal specimens from animals exposed to sludged pastures with demonstrable Salmonella contamination remains suggestive.

Similar reports come from Germany where Hess and Breer (1975) and Hess (1981) claimed numerous cases of salmonellosis in cattle herds were associated with animals grazing in fields where sludge had been previously applied. The strongest evidence cited by these authors arose from a situation in which cattle were fed grass cut from a field 4 weeks after sludge application. Six days later nine animals had diarrhea and fever. Salmonella tokoin was recovered from all nine animals as well as from grass in the sludged field 7 weeks after sludge application. The rarity of S. tokoin and the rapid, coincident onset of salmonellosis was taken as proof of a chain of infection directly attributable to sludge even though the applied sludge itself had not been monitored.

Hess and Breer (1975) provided further retrospective epidemiological data spanning the 6 year period of 1969 to 1974 in support of their earlier observation. Yearly cycles of Salmonella isolation from cattle repeatedly show peak incidences in May (1.5% positive) and August (4% positive) which closely corresponded to sludge application and annual grazing seasons. These authors noted that hogs confined to permanent stalls did not show seasonal increases in Salmonella isolation and only 1.2% of farm manure samples not associated with domestic sludge application yielded Salmonella species (Hess, 1981). However, the occurrence of Salmonella infections in two different species may not be comparable as no data were presented documenting the incidence of Salmonella in hogs pastured in fields. Specifically, overall hygenic conditions may be superior in permanent stalls if they are cleaned regularly.

Undoubtedly, cattle ingesting grass contaminated with viable organisms can become infected. Indeed, early work by Taylor and Burrows (1971) demonstrated that calves grazing on pastureland contaminated with 10^6 S. dublin per ml of dairy waste slurry (0.2% solids) began shedding the organism within 24 to 48 hours after their initial exposure. Notably, when the level of Salmonella contamination was reduced to 10^3 organisms per ml of slurry none of the grazing animals became infected, indicating that their exposure was below an infectious dose.

Among the sludge studies cited above, no controlled experiments comparing infection rates in herds grazing on sludged and unsludged pasturage have been reported. However, the data are suggestive and the length of time between sludge application and animal exposure may provide supplemental information on the survival of Salmonella on sludged fields. In those cases cited above where feeding on sludge-contaminated grass has been implicated in salmonellosis in cattle, the animals were exposed to the contaminated fodder within 3 to 4 weeks of sludge application.

Other Bacteria

Selected studies have focused on other bacterial species because of their presence in sludge, their pathogenic potential, and/or their relative hardiness to environmental extremes. In a preliminary evaluation of sludged fields in Pennsylvania, Kenner and associates (1971) noted the recovery of *Pseudomonas aeruginosa* 16 weeks after the initial application of aerobically digested sludge. Subsequent testing was conducted with anaerobically digested sludge and undigested activated sludge applied to experimental plots in Cincinnati, Ohio, beginning in November. Levels of indigenous *Pseudomonas aeruginosa* were near or below detection limits in the field receiving digested sludge. Variable increases and decreases in *Pseudomonas* levels in activated sludge-amended soil confounded the estimation of organism die off in these plots. However, *Pseudomonas aeruginosa* was consistently recovered from both raw sludge-amended plots for 13 weeks over the winter test period.

Mycobacterium species fulfill all three of the criteria listed above. Their association with disease, especially tuberculosis, and their enhanced potential survival due to their unique cell wall structure are well known. To ascertain the persistence of this group of bacteria, Van Donsel and Larkin (1977) conducted studies in which activated sludge was mixed with *Mycobacterium bovis* BCG strain 1029 (Phipps) and then sprayed onto vegetable plots. Four sludge applications of 2.5 cm each were made at weekly intervals. Soil sampling to a depth of 2.5 cm began 1 day after the last spraying. *Mycobacterium bovis* was isolated from soil through 29 days with a linear T_{90} of 8 days. This unexpectedly rapid inactivation was attributed to the high temperatures recorded during the study (i.e., surface temperatures as high as 45°C were recorded during afternoons).

Although less well known, *Listeria monocytogenes* shares many of the same attributes as other relatively stable bacteria. This bacterium is frequently associated with meningitis and meningoencephalitis in humans and has been isolated from many domestic and wild animal species. Observations based on experimentation in medical laboratories have long suggested the relative stability of this organism under conditions of extreme temperature (i.e., pasteurization) and desiccation (Dubos and Hirsch, 1965). Watkins and Sleath (1981) observed the distribution of this bacterium in waters and sludge-amended land in England. *L. monocytogenes* was enumerated using cold enrichment for a minimum of 1 week followed by selective growth in a nutrient broth containing thiocyanate and nalidixic acid. All isolations were serologically confirmed. Comparative analyses of the same samples showed that *L. monocytogenes* was recovered from sludge at levels equal to or exceeding *Salmonella* sp. and appeared to survive for longer periods of time in sludge-sprayed fields. In one study weekly samples collected after the application of sludge to land showed virtually no loss of *L. monocytogenes* over an 8 week period while indigenous *Salmonella* sp. disappeared with a T_{90} of approximately 11 days. Unfortunately, no climatological information was given for this limited study.

Clostridium represents yet another group of bacteria with pathogenic potential and extended survival capabilities due to its spore-forming ability. The introduction of sewage sludge into soil can substantially increase the levels of Clostridia in this ecosystem. Working on field plots located in the Gembloux region of France, Bourillet and associates (1981) monitored the survival of sulfite-reducing clostridial spores. One 16 m^2 plot received the equivalent of 100 m^3 of sludge (6.5% solids) per hectare while another plot remained in its native state. Several days after spreading, the sludged field was broken up to a depth of 10 cm; however, the dried sludge crust remained mostly as large fragments on the surface. In soil samples collected to a depth of 30 cm and in which some dried sludge was visible, Clostridium recovery was extended. After 12 weeks of sampling, spore levels had decreased by less than one $\log_{10}$. Laboratory studies using columns (25 cm diameter x 30 cm length) packed with sterile sand yielded similar results. The columns were held in a greenhouse during the first month following liquid sludge application (6.6% solids, 100 m^3/ha) to avoid exposure to frost. Thereafter, the columns were transferred outside and exposed to prevailing environmental conditions. During the 12 weeks of these column studies, less than a 0.5 $\log_{10}$ reduction in the number of spores recoverable from the sludge crust was noted. The authors also reported significant levels of Clostridia at the base of grasses growing on sludge-treated fields (51 m^3/ha).

Another study following the fate of Clostridia perfringens was conducted during the summer and fall months at the University of Washington in Seattle (St. John and Matches, 1982). Dewatered, domestic sludge was applied to two tilled plots to a depth of approximately 2.5 cm. Sludge was left undisturbed on the surface of one plot and tilled into the soil to a depth of 15 to 18 cm in the second plot. C. perfringens was enumerated using iron-milk medium in a multiple tube fermentation test; however, no further confirmatory tests were done to verify the specificity of this assay. Soil samples were collected at the surface and at 7.6 and 15 cm depths. Because C. perfringens is ubiquitous in natural ecosystems, organism survival was impossible to ascertain in retilled plots as sludge amendment resulted in less than a one $\log_{10}$ increase in organism recovery per gram of soil when compared with the untilled sludge plot at depths of 7.6 and 15 cm. However, no significant decrease in C. perfringens levels was observed over a 24 week monitoring period in the plots in which sludge had been mixed into the topsoil. C. perfringens in sludge left on the soil surface decayed gradually with an estimated T_{90} of 21 weeks. Such observations are in keeping with the persistence of these Clostridium species reported from the French study and reflect the acknowledged longevity of bacterial spores.

Indicator Bacteria

Since the level of bacterial pathogens in treated sludge can be quite low, many investigators have chosen to use indicator bacterial groups in evaluating the survival of sludge-associated bacteria applied to soils. While inactivation rates of indicator bacteria do not necessarily reflect the survival of human pathogens, available data will be discussed to provide a framework in which to compare the behavior of several bacterial species in sludge introduced into the soil environment.

As part of an early study designed to evaluate the survival of various enteric bacteria in soil, Mallmann and Litsky (1951) seeded either

Streptococcus faecalis (designated as fecal streptococcus for this review) or Escherichia coli (designated as fecal coliform) into cylinders containing a sandy loam soil mixed with sterilized raw sludge in concentrations of 2%, 5%, 10%, or 20%, by weight. The original moisture level was maintained by regularly restoring the initial cylinder weight by the addition of water. Tests were conducted in a greenhouse to insure a constant temperature and to avoid the introduction of extraneous bacteria by animals or birds. Bacteriological examinations were made at weekly intervals for 11 weeks using MPN procedures. With few exceptions the levels of both indicator bacteria increased by as much as 3- to 5-fold between time zero and the 1 week sampling point. Thereafter, fecal streptococci were inactivated at similar rates with T_{90} values ranging from 8 to 14 days, while 99% inactivation occurred within 2 to 4 weeks following sludge incorporation into the sandy loam soil (Table 11, line codes F, G, H, and I). Fecal coliforms died away at a significantly slower rate than fecal streptococci with apparent T_{90} values ranging from 24 to 42 days (Table 12, line codes U, V, W, and X). In addition, in the absence of sludge, fecal coliform bacteria were inactivated at a more rapid rate when compared to the 10% and 20% sludge loadings (Table 12, line codes T, W, and X). Thus the introduction of sludge appeared to extend coliform survival, possibly by moderating desiccation.

The persistence of indigenous fecal coliforms in sludge applied to land was documented by Kenner and associates (1971) at two field sites in Pennsylvania. Anaerobically digested sludge was applied in April, 1968, to a field that had a meadow cover crop. Soil in the test field was a sandy loam with a pH of 6.8. Sludge loading approached 18 t/ha (Cooke, 1969). After application, the fecal coliform level was 2,400 cfu/g. Twenty-one weeks later, fecal coliforms were recovered at a level of 192 cfu/g from this sludged field. A second study site at Hanover, PA, received 7 t/ha of aerobically digested sludge in the same time frame. Clover and grass were growing on the field. The silt loam had a pH of 7.5 (Cooke, 1969). The initial assay of sludge-amended soil yielded 5.5×10^2 fecal coliforms/g. These organisms were still recovered at 18 weeks at a level of 22 cfu/g. Since only a few samples were reported from this portion of the study, the possibility of regrowth accounting for the extended persistence of fecal coliforms cannot be addressed, nor can a T_{90} value be established with any confidence. Furthermore, in the absence of unsludged control fields, the presence of low levels of fecal coliforms cannot be fully interpreted.

To obtain additional information on bacterial survival under winter conditions, anaerobically digested primary sludge and undigested activated sludge were applied to test plots in Cincinnati, Ohio, in November, 1969 (Kenner et al, 1971). Soil samples were monitored weekly for fecal coliforms until June, 1970, by the use of a membrane filtration procedure applied to settled soil supernatants. Despite some variability in fecal coliform levels, graphical interpretation of results showed a T_{90} value of 25 days in the plot receiving anaerobically digested sludge while in the plots receiving undigested activated sludge T_{90} values for fecal coliforms ranged from 32 to 40 days (Table 12, line codes Y, AA, and Z, respectively). Temperatures ranged from below freezing (-19°C) to 16°C during the 3 months immediately following sludge application. Just over 530 cm of precipitation were recorded in this same 3 month period. Since the environmental conditions

TABLE 11. SUMMARY OF FECAL STREPTOCOCCI SURVIVAL IN SLUDGE APPLIED TO SOIL

PART I.

Line Code	Reference	Sludge Type	% Solids	Sludge Application	
				Rate (t/ha)	Mode
A	Berron, et al, 1981	Raw, Thickened +	5.5	6	Spread
B	Berron, et al, 1981	Raw, Thickened +	8.7	9	Spread
C	Berron, et al, 1981	Raw, Thickened +	8.7	18	Spread
D	Yanko, et al, 1978	Digested (Anaerobic)	--	--	Sprayed, tilled
E *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	0 #	Mixed, hoed
F *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	2 #	Mixed, hoed
G *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	5 #	Mixed, hoed
H *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	10 #	Mixed, hoed
I *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	20 #	Mixed, hoed
J	Möller, et al, 1985	Digested (Anaerobic)	--	--	Spread, tilled
K	Möller, et al, 1985	Digested (Anaerobic)	--	--	Spread, tilled
L	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
M	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
N	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
O	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried

PART II.

Line Code	Soil Characteristics			Mean Ambient Temp. (°C)	Initial Microbe Conc. **	Die-off			
						Surface (0-5 cm)		Depth (5-15 cm)	
	Type	Moisture (%)	pH			T_{90}(d)	T_{99}(d)	T_{90}(d)	T_{99}(d)
A	N.I.	--	--	Warm	--	23	--	--	--
B	N.I.	--	--	Cool	--	20	36	--	--
C	N.I.	--	--	Cool	--	22	40	--	--
D	Loam	Dry	--	--	6,000 (a)	28	63	--	--
E *	Sandy Loam	--	--	--	1,700 (b)	7	15	--	--
F *	Sandy Loam	--	--	--	2,200 (b)	12	17	--	--
G *	Sandy Loam	--	--	--	3,200 (b)	8	14	--	--
H *	Sandy Loam	--	--	--	23,000 (b)	14	27	--	--
I *	Sandy Loam	--	--	--	27,000 (b)	10	21	--	--
J	Sand	23	5.2	Warm	10^3 (a)	--	--	30	60
K	Sand	16	6.1	Warm	10^3 (a)	--	--	15	30
L	Heavy Clay	--	--	12 ++	65,000 (c)	--	--	20	40
M	Medium Clay Loam	--	--	12 ++	115,000 (c)	--	--	20	40
N	Light Sand	--	--	12 ++	55,000 (c)	--	--	30	--
O	Light Sand	--	--	15 ++	115,000 (c)	--	--	12	44

N.I. = No Information
* Organisms Added
+ Industrial and Municipal
# Percent Total Weight
++ Soil Temperature

** Units Reported: (a) MPN/g sludged soil
(b) MPN/g sludged soil (dry wt)

TABLE 12. SUMMARY OF FECAL COLIFORM SURVIVAL IN SLUDGE APPLIED TO SOIL

PART I.

Line Code	Reference	Sludge Type	% Solids	Sludge Application	
				Rate (t/ha)	Mode
A	Edmonds, 1976	Digested (Anaerobic)	20-40	461	Sprayed
B	Edmonds, 1976	Digested (Anaerobic)	20-40	307	Sprayed
C	Hagedorn, 1980	Digested Primary (Anaerobic)	6.2	19	Sprayed, tilled
D	Hagedorn, 1980	Digested Primary (Anaerobic)	6.2	19	Sprayed, tilled
E	Hagedorn, 1980	Digested Primary (Anaerobic)	2.5	36	Sprayed, tilled
F	Hagedorn, 1980	Digested Primary (Anaerobic)	2.5	36	Sprayed, tilled
G	Hagedorn, 1980	Digested Primary (Anaerobic)	6.2	12	Sprayed, tilled
H	Hagedorn, 1980	Digested Primary (Anaerobic)	6.2	12	Sprayed, tilled
I	Hagedorn, 1980	Digested Primary (Anaerobic)	2.5	22	Sprayed, tilled
J	Hagedorn, 1980	Digested Primary (Anaerobic)	2.5	22	Sprayed, tilled
K	Berron, et al, 1981	Raw Thickened *	8.7	18	Spread
L	Berron, et al, 1981	Raw Thickened *	5.5	3	Spread
M	Berron, et al, 1981	Raw Thickened *	5.5	6	Spread
N	Yanko, et al, 1978	Digested (Anaerobic)	--	--	Sprayed, tilled
O	Yanko, et al, 1978	Digested (Anaerobic)	--	--	Sprayed, tilled

PART II.

Line Code	Soil Characteristics			Mean Ambient Temp. (°C)	Initial Microbe Conc. **	Die-off			
						Surface (0-5 cm)		Depth (5-15 cm)	
	Type	Moisture (%)	pH			T_{90}(d)	T_{99}(d)	T_{90}(d)	T_{99}(d)
A	Gravelly Glacial Outwash	24-30	5.4	Warm	100,000 (a)	84	165	--	--
B	Gravelly Glacial Outwash	21-23	5.5	Cool	120,000 (a)	64	128	--	--
C	Fine Silt	--	7.1	9 +	80,000 (b)	--	--	7	14
D	Fine Silt	--	5.8	9 +	80,000 (b)	--	--	9	17
E	Coarse Silt	--	6.2	10 +	80,000 (b)	--	--	4	9
F	Fine Clay	--	5.8	9 +	80,000 (b)	--	--	6	12
G	Fine Silt	--	7.1	27 +	330,000 (b)	--	--	28	56
H	Fine Silt	--	5.8	26 +	330,000 (b)	--	--	28	56
I	Coarse Silt	--	6.2	27 +	40,000 (b)	--	--	16	32
J	Fine Clay	--	5.8	27 +	40,000 (b)	--	--	16	32
K	N.I.	--	--	Cool	--	23	34	--	--
L	N.I.	--	--	Warm	--	22	30	--	--
M	N.I.	--	--	Warm	--	23	--	--	--
N	Loam	Moist	--	--	40,000 (b)	35	100	--	--
O	Loam	Dry	--	--	1,000 (a)	15	39	--	--

N.I. = No Information
* Significant Industrial Component
+ Soil Temperature

** Units Reported: (a) MPN/g sludge
(b) MPN/g sludged soil

TABLE 12. SUMMARY OF FECAL COLIFORM SURVIVAL IN SLUDGE APPLIED TO SOIL (Continued)

PART I.

Line Code	Reference	Sludge Type	% Solids	Sludge Application	
				Rate (t/ha)	Mode
P	Johnson, 1979	Dewatered, Dried	30	45	Spread, tilled
Q	Johnson, 1979	Dewatered, Dried	30	90	Spread, tilled
R	Johnson, 1979	Digested (Aerobic), Thickened	70	225	Spread, tilled
S	Carroll & Ross, 1983	Digested (Anaerobic)	--	13	Injected
T *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	0 +	Mixed, hoed
U *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	2 +	Mixed, hoed
V *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	5 +	Mixed, hoed
W *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	10 +	Mixed, hoed
X *	Mallmann and Litsky, 1951	Raw (Sterilized)	--	20 +	Mixed, hoed
Y	Kenner, et al, 1971	Digested (Anaerobic)	--	--	Spread
Z	Kenner, et al, 1971	Raw, Activated	--	--	Spread
AA	Kenner, et al, 1971	Raw, Activated	4	17	Spread
BB	Bergstrom & Langeland, 1981	Raw	20	213	Mixed
CC	Edmonds, 1979	Digested (Anaerobic)	20-40	461	Spread
DD	Möller, et al, 1985	Digested (Anaerobic)	--	--	Spread, tilled
EE	Möller, et al, 1985	Digested (Anaerobic)	--	--	Spread, tilled

PART II.

Line Code	Soil Characteristics			Mean Ambient Temp. (°C)	Initial Microbe Conc. **	Die-off			
						Surface (0-5 cm)		Depth (5-15 cm)	
	Type	Moisture (%)	pH			T_{90}(d)	T_{99}(d)	T_{90}(d)	T_{99}(d)
P	Silt Loam	--	7	16	200 (a)	12	22	--	--
Q	Silt Loam	--	7	16	3,500 (a)	7	12	--	--
R	Silt Loam	14	7.4	16	7,900 (a)	12	21	--	--
S	Heavy Clay	Unsaturated	8	--	920 (b)	--	--	49	--
T *	Sandy Loam	--	--	--	4,400 (b)	20	39	--	--
U *	Sandy Loam	--	--	--	7,900 (b)	28	56	--	--
V *	Sandy Loam	--	--	--	11,900 (b)	24	67	--	--
W *	Sandy Loam	--	--	--	9,700 (b)	42	--	--	--
X *	Sandy Loam	--	--	--	2,700 (b)	42	--	--	--
Y	N.I.	--	--	3	6,100 (c)	25	50	--	--
Z	N.I.	--	--	-1	6,800 (c)	40	80	--	--
AA	N.I.	--	--	1	26,000 (c)	32	64	--	--
BB	N.I.	--	--	--	2,000 (a)	--	--	21 #	38 #
CC	Gravelly Glacial Outwash	--	6.0	--	--	50	75	--	--
DD	Sand	23	5.2	Warm	100,000 (a)	--	--	10	22
EE	Sand	16	6.1	Warm	200 (a)	--	--	10	90

N.I. = No Information
* Organisms Added
+ Percent Total Weight
# Sampled to Depth of 25 cm

** Units Reported:
(a) MPN/g sludged soil
(b) MPN/ml of diluent
(c) cfu/g sludged soil

were identical for all three plots, it appears that fecal coliforms in anaerobically digested sludge were more rapidly inactivated. After 11 weeks on the digested sludge plot, fecal coliforms were no longer detected through the winter period, although sporadic recoveries were again made in March and June, 1970. In undigested sludge plots these indicator bacteria were recovered for about 20 weeks. This apparent enhanced persistence may be attributed, at least in part, to the 10- to 100-fold higher levels of fecal coliforms initially present in the activated sludge-treated plots.

As part of an extensive laboratory study designed to evaluate some of the factors influencing microbial degradation of digested sludge applied to soil, Miller (1974) also noted the relative survival of indicator bacteria over a 6 month period. Anaerobically digested sludge was added to test columns at a rate of either 90 t/ha or 224 t/ha into three soil types: sand, silt loam, and clay. One, 3, and 6 months after application, indicator bacteria including total and fecal coliforms and fecal streptococci were enumerated using membrane filtration techniques. Because of high variability between duplicate column results, the author chose to present the data in terms of relative percent survival in 10 to 20% increments. At 1 month after application, the population of total coliforms and fecal streptococci did not exceed 40% of the levels originally present in all three sludge-amended soil types. Furthermore, their levels continued to decrease with time although both groups of indicators were detected even after 6 months, except total coliforms which had disappeared from the clay soil by the end of the study. Total coliforms persisted equally well in sand and silt loam soils. Fecal streptococci survival was lowest in the silt loam with only 1 of 4 samples positive at 6 months. Fecal coliforms did not exceed 30% of the original levels in sludged soils at 1 month and by 3 months only the silt loam soil yielded these bacteria at a level less than 0.1% of that initially present. In general, a trend for longer bacterial survival was observed in soils incubated under saturated moisture conditions rather than in soils maintained at their field capacity. Fecal streptococci persisted at the highest levels in the clay soil, independent of moisture maintenance. From a practical standpoint, however, the degree of hydration of a clay soil under either field capacity or saturated conditions may be quite similar.

Limited data on the behavior of indicator bacteria in sludge-amended soils was presented by Larkin and associates (1978) in their report on the contamination of vegetables grown in sludged soil. Using standard MPN enumeration, indigenous fecal coliform and fecal streptococcus concentrations in sludged soil exhibited a linear decline through 6 weeks while total coliforms continued to be recovered at "high levels" (no data presented).

Faced with the necessity of disposing of increasing quantities of biological waste sludges, several large municipalities conducted their own monitoring programs to address the impact of sludge disposal on agricultural lands. One of the earliest large-scale studies was carried out by the Metropolitan Sanitary District of Greater Chicago. High rate, anaerobically digested sludge was barged to a 2,900 ha site in Fulton County, Illinois, pumped to holding basins, and finally applied to pastureland that had been previously strip-mined. A large portion of the sludge was incorporated into fields by discing (Lue-Hing et al, 1979). Monitoring for indicator bacteria in sludge-amended soil at the Fulton County disposal site was done using membrane filtration procedures outlined in Standard Methods (O'Brien et al, 1978). Annually two samples from control and test fields were composited and analyzed for total coliforms, fecal coliforms, and fecal streptococci. During the first year of sampling (1975), total coliform counts per gram of dry soil showed little difference, ranging from 2.3×10^2 to 1.1×10^4 in four sludged fields while comparable levels in two control fields were 2×10^3 and 7.1×10^4. Fecal coliform levels were slightly higher in test fields, ranging from 2.2×10^1 to 6.0×10^3 compared to control fields where levels of 8.9×10^0 and 6.5×10^2 were reported. Fecal streptococci were about 10- to 30-fold higher in three of four test fields, with values as high as 3.3×10^4/g (Sedita et al, 1977).

As monitoring continued over an additional two-year period, however, differences between four test fields and a single control field became less notable. For fecal coliforms and fecal streptococci, geometric mean levels (1975 & 1976) in two of the test fields were equal to or less than the control field. Total coliform counts were elevated well above the control in three test fields (Lue-Hing et al, 1979). At the end of the 3 year monitoring program total coliforms and fecal streptococci were elevated 15-fold over the control field in only one test field. It should be noted that this field had received less than 40% of the cumulative sludge application placed on the other three fields, which ranged from 143 to 174 t/ha (O'Brien et al, 1978). This observation coupled with the low level of fecal coliforms on this test field raises the possibility of contamination from other sources. The lack of timely monitoring during the periods prior to and immediately following sludge application precludes the interpretation of these Fulton County data from the standpoint of bacterial survival. However, the results are consistent with the conclusion that within the limits of the sampling and detection methodology used, no significant long-term buildup of indicator bacteria could be attributed to the application of anaerobically digested sludge.

A similar monitoring approach was used by the Los Angeles/Orange County Metropolitan Area in cooperation with the University of California at Riverside. Field demonstration sites were set up in which anaerobically digested sludges or dewatered, composted sludges were applied to loam soils at application rates of 0, 23, 45, and 90 t/ha/yr (Yanko et al, 1978). During the first growing season under study, two digested sludges were applied to test plots on two different soil series in May, 1976 (Yanko et al, 1976). Approximately 2 weeks later after the sludge layer had dried partially, the fields were disced and sludged soil samples were analyzed using Standard Methods MPN procedures. Levels of total coliforms and fecal coliforms were higher than expected, hence a value of greater than 2.4×10^4/g was reported. This level was 4- to 1,000-fold higher than control plots with respect to total coliforms and as much as 10^3 to 10^4 higher for fecal coliforms. The addition of these anaerobically digested sludges did not increase fecal streptococci levels as dramatically. Even at a loading of 90 t/ha/yr, test plots contained a maximum of 1.3×10^3 MPN/g reflecting only a 65-fold increase over the corresponding control plot. By harvest time in October, 1976, it appeared that levels of fecal coliforms had stabilized near or below levels in control plots for each test series; i.e., little difference was observed in fecal coliform numbers regardless of the initial sludge loading with values generally ranging from 10 to 50 MPN/g. While total coliform and fecal streptococcus levels had likewise stabilized, a few test plots contained from 10- to 100-fold higher organism concentrations than corresponding controls. However, results for total coliforms are difficult to interpret as three of four control plots had elevated coliform concentrations at 1.2 x 10^4 to 1.2 x 10^5 MPN/g of dry soil; levels which were 50- to 100-fold above the same plots in June.

Similar testing was subsequently initiated with the application of digested sludges in January and June, 1977 (Yanko et al, 1978). The application of digested liquid sludge to agricultural fields in the winter led to increases in the number of indicator bacteria recovered from the treated soils which were sampled within 1 month of treatment. Total coliform levels in plots receiving 90 t/ha/yr were elevated 2- to 50-fold over control plots reaching levels of 5 x 10^5 to 1 x 10^6 MPN/g of dry soil. Increases in fecal coliforms were more dramatic in three of four experimental plots (160- to >2,000-fold elevation) since background levels were low; however, actual organism concentrations approached 3 x 10^3 MPN/g. Fecal streptococcus levels after sludge application were 4 to 10 times higher than control plots, with the highest reported concentration being 1 x 10^3 MPN/g. Six months later, maximal reported levels in the same experimental plots were 8.4 x 10^4 total coliforms, 4.5 x 10^1 fecal coliforms, and 2.7 x 10^2 fecal streptococci/g of dry soil. Soil samples were not analyzed after the application of sludges in June, 1977. By October, 1977, when soils from these summer plots were analyzed, indicator bacteria levels in sections receiving 90 t/ha/yr were near or below levels in control plots with the exception of fecal coliforms in one test plot where levels of 2.1 x 10^2 organisms/g of soil were recorded, 30-fold higher than the parallel control plot. Thus, while this 2 year study documented short-term increases in indicator bacteria as a result of sludge application to soil, these bacterial populations were reduced in most cases to background levels within 4 to 6 months at this California site.

In an attempt to define the kinetics of microorganism survival, Yanko and associates (1978) also conducted smaller scale studies using a more frequent sampling schedule. Thirty cm of anaerobically digested sludge was applied to a loam field over a 3 day period in June, 1977, allowed to dry, and rototilled into the soil. Steel rings, measuring about 46 cm h x 46 cm d, were driven into the soil and various experimental conditions were maintained within the encircled area. Sludged and control plots were either kept natural or moisture tension was maintained between 0.2 to 0.8 bar with or without a cover crop. Surface soil to a 5 cm depth was analyzed for indigenous bacteria using MPN procedures. Initial samples were taken immediately after tilling and at subsequent 4 week intervals. In virtually every case bacterial numbers were higher at 4 weeks than after tilling.

In the dry soil, fecal and total coliforms showed a linear decline from 4 weeks through 12 to 16 weeks with essentially identical T_{90} values of 16 days (Table 13, line code C) and 15 days (Table 12, line code 0), respectively. Fecal streptococci decreased to near background by 8 weeks, with an apparent T_{90} of 28 days (Table 11, line code D). After 12 weeks, however, all indicator bacteria were observed to increase in numbers through 16 weeks

TABLE 13. SUMMARY OF TOTAL COLIFORM SURVIVAL IN SLUDGE APPLIED TO SOIL

PART I.

Line Code	Reference	Sludge Type	% Solids	Sludge Application Rate (t/ha)	Sludge Application Mode
A	Edmonds, 1976	Digested (Anaerobic), Dewatered	20-40	307	Spread
B	Edmonds, 1976	Digested (Anaerobic), Dewatered	20-40	461	Spread
C	Yanko et al, 1978	Digested (Anaerobic)	--	--	Sprayed, tilled
D	Wallis et al, 1984	Digested (Anaerobic), Lagooned	--	--	Injected
E	Wallis et al, 1984	Digested (Anaerobic), Lagooned	--	--	Injected
F	Wallis et al, 1984	Digested (Anaerobic), Lagooned	--	--	Spread
G	Sekla and Stackiw, 1983	Digested (Anaerobic)	--	--	Injected
H	Carroll and Ross, 1983	Digested (Anaerobic)	--	13	Injected
I	Carroll and Ross, 1983	Digested (Anaerobic)	--	13	Injected
J	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
K	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
L	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried
M	Dickson and Tribe, 1986	Raw +	5	20	Mixed, buried

PART II.

Line Code	Soil Characteristics			Mean Ambient Temp. (°C)	Initial Microbe Conc. **	Die-off			
						Surface (0-5 cm)		Depth (5-15 cm)	
	Type	Moisture (%)	pH			T_{90}(d)	T_{99}(d)	T_{90}(d)	T_{99}(d)
A	Gravelly Glacial Outwash	21-23	5.5	Cool	1E6 (a)	129	260	--	--
B	Gravelly Glacial Outwash	24-30	5.4	Warm	1E6 (a)	172	350	--	--
C	Loam	Dry	--	--	10,000 (b)	16	28	--	--
D	N.I.	--	--	Warm	1E9 (b)	25	50	--	--
E	N.I.	--	--	Cool	500,000 (b)	40	--	--	--
F	N.I.	--	--	Cool	1E8 (b)	140	--	--	--
G	Heavy Clay	--	--	--	2,200 (c)	--	--	42	--
H	Heavy Clay	Unsaturated	8.0	--	2,200 (c)	--	--	35	--
I	Heavy Clay	Unsaturated	8.0	--	48 (c)	--	--	70	--
J	Heavy Clay	--	--	12 *	4.0E7 (c)	--	--	14	28
K	Medium Clay Loam	--	--	12 *	1.8E7 (c)	--	--	16	32
L	Light Sand	--	--	12 *	2.3E7 (c)	--	--	16	32
M	Light Sand	--	--	15 *	1.1E7 (c)	--	--	9	18

N.I. = No Information
* Soil Temperature
+ Significant Industrial Component

** Units Reported: (a) MPN/g sludge
(b) MPN/g sludged soil
(c) MPN/ml of diluent

(fecal streptococci) to 20 weeks (total and fecal coliforms), providing evidence for bacterial regrowth.

In moist soils results were more difficult to interpret. The number of indicator bacteria on control plots showed elevated levels at various times during the 20-week monitoring period, reaching levels at or above sludged fields. Total coliforms and fecal streptococci did not show any consistent decrease over the course of this 5-month study. Fecal coliform disappeared through 8 weeks with an apparent T_{90} of 35 days (Table 12, line code N), but organism dieaway slowed considerably thereafter, resulting in an estimated T_{99} of 100 days.

While relatively rapid bacterial inactivation has been documented when sludges were applied to soils and subsequently exposed to hot and drying environmental conditions, the fate of sludge-associated organisms exposed to cooler conditions is less well studied. To evaluate the feasibility of placing sludges on land in cold climates, aerobically digested, dewatered sludge was applied to tilled, silt loam farmland at the University of Alaska's Agricultural Experiment Station near Fairbanks (Johnson, 1979). After being spread on the soil surface, the sludge was rototilled to a depth of 20 cm. In the field receiving a loading of 225 t/ha, thickened sludge had been on a drying bed for 6 months prior to application. Sludge used on plots receiving 45 and 90 t/ha had been mechanically dewatered using a polymer coagulant and air dried for about 4 months. Fecal coliforms were enumerated at the soil surface using the Standard Methods MPN procedure. The plots receiving digested, dewatered sludge which had been air dried for only 4 months carried a higher load of fecal coliforms despite the lower loading rates, resulting in 200 MPN/g in the 45 t/ha plot and 3,500 MPN/g in the 90 t/ha plot at the time of application (Table 12, line codes P and Q). By 21 days post-application, fecal coliform levels were below 15 MPN/g with estimated T_{90} values of 12 and 7 days. Similar levels of fecal coliforms were observed within 28 days after the application of 225 t/ha of air-dried sludge. In this experimental test series a T_{90} value of 12 days was also estimated (Table 12, line code R). Since the study was conducted during late summer and early fall, the temperatures were quite moderate despite the northern location of the site. Over the first 30 days of monitoring, temperature at the soil surface ranged from about 21°C to 38°C with temperatures at a depth of 15 cm recorded as 15°C to 22°C. Soil moisture was given as 14% for both the unsludged control plot and the plot receiving 225 t/ha of air-dried sludge.

Working over a period of several years, Edmonds (1976) monitored the effects of applying sludge to forested regions in the Pacific Northwest. Anaerobically digested, dewatered sludge (20% to 40% solids) was surface spread over either forested or clearcut areas within the University of Washington's Pack Forest near Eatonville, Washington. Using Standard Methods MPN procedures, total coliform and fecal coliform bacteria were monitored 5 cm below the sludge surface, in the soil 5 cm beneath the sludge-soil interface, and 5 cm beneath the soil-litter interface in control plots. Exceedingly high variability was documented for indicator analyses with standard errors of 50% to 100% of the recorded coliform values frequently reported. This may, in part, be due to the nature of the test sites where sludge was spread over a very gravelly glacial outwash surface. Under these conditions uniform sludge application and representative sampling could be difficult.

In clearcut areas at this study site, coliform bacteria exhibited longer survival following sludge application in the summer than in the winter. Specifically, T_{90}/T_{99} values for total coliforms in the sludge layer were estimated at 129 days and 260 days following a January, 1974 application and 172 days and 350 days after the July, 1974 sludge spreading (Table 13, line codes A and B, respectively). Similarly, 10% of the fecal coliforms in the sludge survived 64 days after the winter application while 90% inactivation required 84 days during the summer (Table 12, line codes B and A). Notably, the sludge moisture profile for the summer test remained above 40% throughout the survival period while the winter sludge showed a lower moisture (15 to 30%), possibly due to the high sand content of the sludge (Edmonds, 1976). During the following summer, fecal coliforms were again monitored in dewatered sludge which had been applied to a forested plot (Edmonds, 1979). Initial organism dieaway was more rapid during this trial with an estimated T_{90} of 50 days and a T_{99} value of 75 days (Table 12, line code CC).

In parallel studies where increasing quantities (i.e., depths) of sludge were applied to clearcut and forest areas in the summer and winter, similar trends of fecal coliform survival were observed (Edmonds and Mayer, 1981). As measured by the level of organisms surviving 3, 12, and 24 months after sludge application, the most rapid fecal coliform dieaway occurred following the winter application with levels near background by 12 months in all clearcut plots and in two of three forested areas. After a summer sludge application, fecal coliform levels remained elevated at 12 months in all clearcut plots and in two of three forested test areas. By 24 months post-application, fecal coliform levels had returned to background levels. Once again fecal coliform numbers were generally more than two to three times lower in forested plots by 3 months when compared to the comparable loadings in the clearcut area, suggesting a less favorable environment.

In another sludge disposal study in the Pacific Northwest, anaerobically digested sludge was applied to four different soils in an area near Corvallis, Oregon (Hagedorn, 1980). Two soil series, Amity and Woodburn, were classified as fine-silt; one soil series, Cloquato, as coarse silt; and the final soil series, Dayton, as a fine clay. The liquid sludge was applied by spraying and the fields were subsequently tilled to a depth of 15 cm. One test series started with sludge application in December, 1977, with sampling continuing through the end of March, 1978 (winter). The summer study commenced in July, 1978, with sampling through October, 1978. Fecal coliforms were enumerated in surface soil at weekly or biweekly intervals using the Standard Methods MPN procedure.

The winter sludge application resulted in initial fecal coliform levels of about 8×10^4 MPN/g of soil. The overall bacterial inactivation rates were remarkably similar for all four soil types with initial T_{90} values ranging from 4 to 9 days (Table 12, line codes C, D, E, and F). Between weeks 12 and 13, fecal coliforms had declined to $\leq 2 \times 10^1$ MPN/g. Sporadic elevations of fecal coliforms frequently associated with storm events were noted throughout the winter monitoring period. The author attributed these increases to either disaggregation of cell clumps by rainfall or alternatively to fecal coliform regrowth under more moist conditions. A total of 58 cm of rain was recorded over the 3 month winter test. Winter maximum air temperatures exceeded 15°C on only 2 days and were never below 0°C, with most readings falling between 5 to 10°C. Soil temperature recorded at a depth of 5 cm was relatively constant during the winter, rarely exceeding 10°C and never dropping below 1°C.

During the summer test, initial organism counts after sludge incorporation ranged from 4×10^4 to 3.3×10^5 fecal coliforms/g of soil. Levels of these bacteria fluctuated during the first 3 to 4 weeks of this study period with some organism growth occurring. Therefore, inactivation rates were estimated using data collected beginning in August. Fecal coliforms died off in the Cloquato and Dayton soils with an approximate T_{90} of 16 days in late summer to early fall (Table 12, line codes I and J). Using the same approach, T_{90} for the fecal coliforms in both of the fine silt soils was 28 days (Table 12, line codes G and H). By the end of the summer sampling, there were $\leq 3 \times 10^1$ fecal coliforms/g of soil for the Cloquato and Dayton series while levels in the Amity and Woodburn soils exceeded 100/g. Relatively small amounts of rainfall (less than 15 cm) were recorded during the summer monitoring period. Most of the maximum air temperatures during summer sampling were between 23 to 28°C, although during several periods in late summer temperatures in excess of 37°C were recorded. Low air temperatures of 17 to 22°C were common. Large daily fluctuations of as much as ±5°C were observed in summer soil temperatures. Maximum soil temperatures were high in July and early August (30 to 35°C), dropping somewhat by early fall (20 to 25°C). Minimum soil temperatures averaged 15 to 20°C in early summer, decreasing to 10 to 15°C later. The more extended survival of fecal coliforms in the fine silt soils during the summer may have been due to the ability of these soils to retain moisture relative to the coarser textured soils. Overall, the results of this Oregon study confirm the observations previously described for the Washington Pack Forest study. Fecal coliforms disappeared more rapidly in the cool and wet conditions prevailing during the winter test. However, the investigator could draw no statistical correlation between climatic data and die-away rates (Hagedorn, 1980).

In a Norwegian study designed to follow the survival of selected microbial pathogens, Bergstrom and Langeland (1981) also monitored the inactivation of indigenous fecal coliforms using an MPN procedure. It should be noted that in addition to the sewage sludge, pig feces and a laboratory broth culture of Salmonella typhimurium were also mixed into the test plots. Seven of 10 plots were subsequently planted with vegetable crops and regularly watered. From an initial concentration in excess of 10^5 fecal coliforms/100 g of sludge-amended soil, 90% inactivation required 21 days with only 1% of these bacteria remaining at 38 days (Table 12, line code BB).

Working in France, Berron and associates (1981) monitored the fate of fecal streptococci and fecal coliforms following the surface application of thickened sludge to either pastureland or a fallow field in autumn (October) and in winter (January). Evaluation of initial die-off rates for this study was not possible since the first sampling of sludge-amended soil was not done until 15 days after its application. Heavy rains during the first weeks of the fall test series caused considerable dissemination of sludge from the soil surface, thus confounding data interpretation. Based on bacterial levels observed beginning at 15 days and continuing through 3 months, virtually no difference was observed in the rate of fecal streptococcus or fecal coliform inactivation in either test series. As evidenced by the T_{90}/T_{99} values given in Tables 11 (line codes A, B, and C) and 12 (line codes K, L, and M), only 10% of the indicator bacteria were recovered by 23 days. Ninety-nine percent inactivation of fecal streptococci was observed by 40 days, while similar decreases occurred within 34 days for fecal coliforms. It should be noted that in the autumn test series there was evidence of fecal streptococcus regrowth in samples collected from the sludge-treated field through 1 month; then the organisms dramatically disappeared over the next 20 days. No significant differences were noted in the rate of organism decay as a function of sludge loading. Indicator bacteria levels in sludge-amended fields returned to the levels observed in untreated fields within 3 to 4 months after application.

A short-term but broad-based study to evaluate the impact of subsurface sludge injection was conducted by the City of Winnipeg in Manitoba, Canada. An overview of selected results was presented by Sekla and Stackiw (1983) while a comprehensive report detailing the project was submitted by Carroll and Ross (1983). Raw sludges (including waste activated) from two treatment plants as well as one anaerobically digested sludge were injected into heavy clay soils to a depth of about 15 cm. Sludge application at a rate equivalent to 13 t/ha occurred monthly from June until September 1981 into individual test fields. Soil samples were collected within and below the injection trench at depths of 7.5, 15, 30, and 45 cm. Sampling for total and fecal coliforms occurred 1 week, 1 month, 3 months, and 1 year after sludge injection.

Raw sludge from the South End Water Pollution Control Centre was almost exclusively domestic in origin and appeared to carry the highest load of microorganisms. This observation is consistent with the fact that the second treatment plant, North End, received input from industrial users. Since similar survival patterns were observed for indicator bacteria regardless of sludge type, the pattern of organism inactivation in plots receiving raw sludge from the South End plant was considered representative. Although the injection process was subject to some variability, the 15 cm depth within the injection trench generally yielded the highest counts of indicator bacteria reflecting the entrapment of the applied sludge. Without exception the dilution series used to enumerate total and fecal coliforms at the initial 1 week time point were inadequate to determine a valid organism concentration in raw sludge injected soil. Thus, "greater than" values were reported. Relative to background samples collected immediately prior to injection, however, introduction of sludge increased total and fecal coliform levels by a factor of at least 10^4 to 10^5. Total coliform levels were similarly elevated 1 month after injection regardless of the month in which sludge was injected. Three months after sludge injection, total coliform levels at the 15 cm depth in the injection trench were near or below initial background levels, suggesting a reasonably rapid dieaway of at least four $\log_{10}$ units in 3 months. Results observed for fecal coliforms followed a similar pattern, although for sludges injected in June and August, 1981, bacterial levels were reduced by as much as one $\log_{10}$ by 1 month after injection. Once again background levels were approached by 3 months following sludge application and no fecal coliforms were recovered from the injection site 1 year later.

Since the anaerobically digested sludge carried a lower number of organisms, bacterial levels were established during baseline sampling at 1 week, and inactivation rates for total and fecal coliform bacteria in the injection trench could be estimated. When digested sludge was injected in June, 1981, an elevation in total coliform levels was observed at 1 month reflecting a 50% increase over numbers reported at 1 week. Thereafter total coliform died off with an estimated T_{90} of 70 days (Table 13, line code I). Interestingly, fecal coliforms were not detected in the 1 month sample but were recovered at 3 months at a level comparable to that observed in the same time frame in several raw sludge amended plots. After the August, 1981 injection, neither total nor fecal coliforms exhibited an increase in numbers in the entrenched sludge based on comparisons of the soils analyzed at 1 week and 1 month. Inactivation rates of both indicators were similar with an apparent T_{90} of 35 days for total coliforms (Table 13, line code H) and 49 days for fecal coliforms (Table 12, line code S). Data from the Winnipeg study were presented in a slightly different form by Sekla and Stackiw (1983), who reported minimum and maximum levels observed in soils injected with digested sludge. Using only the maximum MPN values given for total and fecal coliforms, T_{90} values of 42 days (Table 13, line code G) and 49 days, respectively, were established in good agreement with estimates based on the report by Carroll and Ross (1983). It can be speculated that the T_{90} of 70 days established for total coliform in sludge applied in June, 1981 actually reflects the die-off rate of a select regrowth population of bacteria.

To compare the relative survival of various sludge-associated microorganisms, anaerobically digested, lagooned, municipal sludge was either surface applied to a grass plot or injected into an agricultural field at sites near Calgary, Alberta, Canada (Wallis et al, 1984). A control hayfield and pastureland grazed by cattle were located over 16 km from the test plots and were presumed to pose a negligible health risk in their native state. While emphasis was placed on the occurrence of parasitic forms, some results reflecting the behavior of coliform bacteria were presented. Samples for analyses were taken from the top 10 cm of the soil profiles and suspensions of composite samples were either spread-plated or inoculated into selective broth media. After the initial sludge injection, two discing events were recorded; however, different dates were reported: 7 days and 55 days in one instance, and 11 days and 75 days in another. Regardless of the dates of the second soil disturbance event, different inactivation curves were observed for the initial bacterial decay during the summer months (June to August) when compared to the late fall and winter conditions (October to December). Total coliform levels in the injected field were reduced by 4 $\log_{10}$ 66 days after summer application with an approximate T_{90} of about 25 days (Table 13, line code D). In the late fall, maximal total coliform levels were reduced with an apparent T_{90} of about 40 days (Table 13, line code E). Similar observations were made during the summer for total coliforms present in sludge simply applied to the soil surface. Within 6 weeks after sludge application, total coliforms had decreased over 2 $\log_{10}$. However, at the next sampling time (ca. 12 wks after sludge application) the level of these indicator bacteria had increased by 1 $\log_{10}$, presumably as a result of regrowth since this pasture was not disturbed by tilling. Thereafter, from October through January a much slower inactivation rate with an estimated T_{90} of about 140 days was observed (Table 13, line code F). The behavior of oxidase negative bacteria growing on MacConkey's agar (presumptive Enterobacteriaceae) paralleled the survival of total coliform bacteria. The cooler and more moist environmental conditions during the fall presumably contributed to this extended survival in both surface spread and injected fields. During the summer and fall, total coliform levels maintained a steady state in control fields. Peak levels of these indicator bacteria in control plots occurred in the early summer (May) with recorded levels reaching 10^8 to 10^9 coliforms/g of soil.

Additional data from the Calgary study were reported by Lehmann and associates (1983). Fecal coliform levels in sludged fields were reported quarterly, thus foregoing timely conclusions of bacterial inactivation. It should be noted, however, that fecal coliforms were generally recovered in the warmer months, even in fields that had received sludge 2 to 3 years earlier. Control fields and pastureland likewise yielded fecal coliforms in spring and summer. The percentage of total coliforms identified as Escherichia coli, Citrobacter freundii, and Enterobacter aerogenes were similar between sludged and control fields. Levels of these bacteria were twice as high on grazed pasture frequently due to the prevalence of the Enterobacter/Klebsiella species. Interestingly, E. coli was not found on sludged fields when 20 to 40 bacterial colonies were identified. However, E. coli was recovered from control fields, most frequently from pastureland grazed by cattle.

Indicator bacteria were also monitored after the application of undigested sludge to a series of test plots (4.8 x 9.6 m) in Oklahoma (Ibiebele et al, 1985). At 3 month intervals beginning in January, 1981, individual plots received either 760, 1520, or 2280 L of sludge, which was subsequently tilled into the subsoil. A control plot received 1520 L of tap water at the time of sludge application. Using a 10% suspension of sludge-soil mix, fecal coliforms and fecal streptococci were enumerated using a membrane filtration procedure. Although composite surface samples were analyzed at 10 day intervals over an 8 month period, grouped results were presented at only five time points. Fecal coliform levels in both the control and test plots showed elevations during the spring and summer sampling. Maximally, two-fold higher levels of fecal coliforms were reported for sludged fields when compared to the unsludged control. Thereafter, fecal coliform levels in test plots were equal to or less than the control field. Results shown for fecal streptococci were more erratic. Background levels in the control plot increased from January through September. As with fecal coliforms, levels of fecal streptococci were only two-fold greater in sludge-amended soil within 90 days of initial application. Thereafter, no significant differences between test and control plots were obvious. Results presented in this paper contribute little to elucidation of organism survival, although taken at face value regrowth of fecal organisms may have occurred.

In a field study conducted in Potsdam, German Democratic Republic, Møller and associates (1985) monitored two agricultural fields receiving mesophilically digested sludges during April, 1981, and May, 1982. Samples of this sand-based soil were collected to a depth of 25 cm. A total of three composite samples were collected at each sampling time and analyzed for fecal coliforms (on lactose-fuchsin agar with confirmation in lactose broth) and enterococci (on sodium-azide nutrient agar). During the first field study, enterococci (classified as fecal streptococci for this review) demonstrated a linear dieaway with a T_{90} of 15 days (Table 11, line code K). However, the following year a more prolonged inactivation with an estimated T_{90} of 30 days was observed (Table 11, line code J). Initial fecal coliform inactivation was identical both years with T_{90} values of 10 days (Table 12, line codes DD and EE). However, remaining fecal coliforms were inactivated more slowly in 1981 as evidenced by a T_{99} of 90 days (Table 12, line code EE). Notably, initial coliform levels were relatively low during 1981 when compared to 1982 and may have already represented a more persistent population. Additionally, since unsludged fields were not monitored, these low levels may have represented indigenous microflora.

As part of a laboratory study designed to monitor the survival of bacterial pathogens in injected sludge, Dickson and Tribe (1986) also reported inactivation data for fecal streptococci (isolated on Slanetz-Bartley agar) and presumptive coliforms (isolated on MacConkey agar at 37°C). Raw sludge was added to three soil types held in glass tubes (15 cm x 2.5 cm diameter) and subsequently buried at a depth of 20 to 35 cm. Soils were held at 9°C, 12°C, and 15°C as previously described. Samples were removed at weekly intervals over an 8 week period. Applying linear regression analysis, Dickson and Tribe (1986) arrived at a T_{90} of 26 days for fecal streptococci and 18 days for coliforms regardless of soil type or temperature. Using a graphical analysis of available data as described for this review, fecal streptococci were inactivated with a T_{90} of 20 days in both clay and loam soils at all temperatures and in sand at 9°C (see Table 11, line codes L and M, for representative data). In sandy soil held at 12°C, fecal streptococci dieaway was slower as evidenced by an approximate T_{90} of 30 days (Table 11, line code N), while more rapid initial inactivation at 15°C was indicated by a T_{90} of 12 days (Table 11, line code O). A similar trend was observed for total coliforms with T_{90} values generally ranging from 14 to 16 days in clay and loam soils at all temperatures and in sand at 9°C (Table 13, line codes J, K, and L). Once again, a more rapid inactivation at 15°C in sand could be estimated graphically with a T_{90} of 9 days (Table 13, line code M). Had a wider range of temperatures been evaluated, the suggestion of differential bacterial inactivation in these different soils could have been more rigorously tested.

Viruses

Attempts to isolate indigenous viruses at sludge application sites have largely yielded no information on the rate of viral decay. Moore and associates (1978) reported the recovery of indigenous viruses at a level of 1.1 plaque-forming unit (pfu)/g of sludged soil collected at a sludge injection site in Montana. The last recorded injection of thickened, raw sludge had occurred in October, 5 to 6 months before the positive recoveries were made.

Viruses were recovered beneath the soil surface and from dry sludge on the surface at both the Montana site and a site injecting digested sludge in Colorado. No conclusions can be made concerning viral inactivation, however, since neither the injected sludges nor the fields were sampled at the time of application.

As part of the field demonstration sites at Riverside, California, Yanko and co-workers (1978) also analyzed soil samples from sludge-amended fields for their viral content. No viral isolates were associated with the disposal of digested sludges, even though viruses were detected in sludges applied to fields in June, 1977. However, these researchers noted that selected viral samples were toxic to the cell culture system used for viral isolation, probably due to high pesticide concentrations in one of the two test fields. In addition, amended soils were sampled 4 to 5 months after sludge application. During a special study in which sampling of a smaller plot was done at monthly intervals, viruses were recovered from three of three sludge amended soils 22 days after sludge addition. Thereafter, only one other positive viral isolation was made throughout a 20 week monitoring period.

Similarly, in a study conducted by the City of Winnipeg, Manitoba, Canada, only one positive viral isolation was recorded over a 1 year monitoring period (Carroll and Ross, 1983). One of four samples taken within the injection trench 1 week after the application of raw sludge yielded virus. Presumably, the recovery procedures involving viral elution and concentration with isoelectric casein (pH 9) were adequate, as positive viral isolations were made from raw sludges prior to land application.

Monitoring for indigenous viruses was attempted at field sites near the city of Calgary, Alberta, Canada (Wallis et al, 1984). Lagooned sludge was either surface applied or injected. No viruses were isolated from the sludge or any test or control fields. Once again the method of viral recovery using elution and concentration in isoelectric casein was adequate for the recovery of viruses from raw and digested sludges. However, sample toxicity may also have contributed to the negative results observed in this study.

As part of a laboratory study of microbial transport in sludge-amended soil, researchers at Metropolitan Seattle monitored dewatered sludge applied to soil cores before and after a 2.5 week experimental period (Andrew Heyward, personal communication, 1983). In the intervening time, the cores were subjected to three cycles of water application (15 cm over 48 hours) followed by drying (5 days). A total of 7 viruses per 100 g of sludge were recovered when the study began and 12 viruses per 100 g of sludge were isolated 17 days later. Thus, under these conditions and at room temperature, no viral inactivation was observed.

To date only three reports of indigenous virus isolation have provided limited data from which viral inactivation rates can be estimated. In the first of these studies, sludge was not applied to land in the normal sense. Using methods optimized for the recovery of poliovirus from activated sludge, Hurst and associates (1978) monitored the survival of indigenous viruses in sludge drying piles located near a treatment plant in Houston, Texas. Three or four samples were collected over each 7 day period. In late summer

(August to September, 1977) viruses were rapidly inactivated with ≤1 pfu/10 g of dried sludge recoverable after 1 week. Estimated T_{90} values for two sequential monitoring periods were 4 days (Table 14, line codes J and K). No viruses were detected in dried sludge after 3 months in the field. Moderate rainfall was recorded only during the second period and ambient temperatures ranged from 20°C to 31°C. Internal temperature in the drying sludge piles was recorded at 27°C just prior to noon on sampling days and probably increased further by late afternoon.

A study of indigenous sludge-associated viruses during lagooning and after the application of lagooned sludge to agricultural land was conducted at the Jay Agricultural Research Center in north central Florida (Farrah et al, 1981). After sludge was applied to land in October, 1979, a very rapid decline in the number of recoverable viruses occurred with an estimated T_{90}/T_{99} of less than 1 day and 5 days, respectively (Table 14, line code L). Viruses were recovered through 9 days following sludge spreading on the soil surface. Although no climatological data were reported, temperatures in Florida should be similar to those observed in the Houston study.

Finally, Møller and associates (1985) isolated indigenous viruses from sludge-amended soils in the German Democratic Republic. As described previously, digested sludges were applied to tilled fields in April, 1981, and in May, 1982. Composite soil samples (10 g each) were eluted with Hanks' salt solution, treated with antibiotics, and ether-extracted at pH 8.5. After centrifugation to remove particulates, the supernatant was recovered, adjusted to pH 7.0, and assayed in the human amnion cell line, Fl, under liquid medium. Infected cultures were maintained for 30 days with as many as two passages. Viral isolates, discerned by cytopathic effect, were identified using type-specific hyperimmune rabbit sera prepared using reference virus strains. Using this procedure, a variety of enteroviruses were detected in 75% of the sludged-soil samples (80 t/ha) set up in the laboratory to test the efficacy of the recovery methods. Total virus values, calculated as the MPN of cytopathic units (MPN cpu), fell between 0.26 cpu/g in loam soil receiving dried sludge and 0.6 cpu/g in sandy soil receiving liquid sludge. Notably, since concentration procedures were not employed, detection sensitivities were limited to 0.1 MPN cpu/g of soil.

Initial monitoring for enteroviruses at the field site that received sludge in 1981 yielded 0.8 MPN cpu/g of soil. Viral inactivation was relatively rapid with an estimated T_{90} of 12 days (Table 14, line code M). After 2 weeks, viral isolation was sporadic at or below 0.1 MPN cpu/g although detections were made through 130 days as interpreted from graphical data. Coxsackievirus B3 and various poliovirus types were recovered during this test series. In 1982, only low levels of poliovirus 1 (≤ 0.1 MPN cpu/g) were recovered initially from sludged fields, therefore an inactivation rate could not be estimated. Curiously, higher numbers of viruses were recovered at much later times with a maximum of 0.57 MPN cpu/g isolated in August, 100 days after sludge application. Viruses were recovered sporadically throughout the 13 month study period and included Coxsackieviruses B2 and B4, echoviruses 3 and 7, and adenovirus 2. Whether these "long-lived" isolates were sludge-associated pathogens surviving at deeper soil depths or whether

TABLE 14. SUMMARY OF SURVIVAL OF VIRUSES IN SLUDGE APPLIED TO SOIL

PART I.

Line Code	Reference	Sludge Type	% Solids	Sludge Application	
				Rate (t/ha)	Mode
A *	Damgaard-Larsen, et al, 1977	Digested (Anaerobic), Dewatered +	22	22	Spread, mixed
B *	Damgaard-Larsen, et al, 1977	Digested (Anaerobic), Dewatered +	22	22	Spread, mixed
C *	Damgaard-Larsen, et al, 1977	Digested (Anaerobic), Dewatered +	22	22	Spread, mixed
D *	Bitton, et al, 1984	Digested, Lagooned	3	8	Spread, mixed
E *	Bitton, et al, 1984	Digested, Lagooned	7	18	Spread, mixed
F *	Bitton, et al, 1984	Digested, Lagooned	7	18	Spread, mixed
G *	Tierney, et al, 1977	Raw, Waste Activated	12	31	Sprayed, hoed
H *	Tierney, et al, 1977	Raw, Waste Activated	12	31	Sprayed, hoed
I *	Tierney, et al, 1977	Raw, Waste Activated	12	31	Sprayed, hoed
J	Hurst, et al, 1978	Digested (Aerobic), Dewatered	--	--	--
K	Hurst, et al, 1978	Digested (Aerobic), Dewatered	--	--	--
L	Farrah, et al, 1981	Digested, Lagooned	--	--	Spread
M	Möller, et al, 1985	Digested (Anaerobic)	--	--	Spread, tilled

PART II.

Line Code	Soil Characteristics			Mean Ambient Temp. (°C)	Initial Microbe Conc. **	Die-off			
						Surface (0-5 cm)		Depth (5-15 cm)	
	Type	Moisture (%)	pH			T_{90}(d)	T_{99}(d)	T_{90}(d)	T_{99}(d)
A *	Sand	--	6	.5	100,000 (a)	--	--	30	60
B *	Sandy Clay	--	7.4	.5	50,000 (a)	--	--	56	100
C *	Sand	--	4.8	.5	10,000 (a)	--	--	30	60
D *	Fine Sand	Moist	--	26 #	40,000 (b)	3	7	--	--
E *	Fine Sand	Dry	--	18-27 #	200 (b)	7	--	--	--
F *	Fine Sand	Dry	--	18-27 #	300 (b)	2	--	--	--
G *	N.I.	--	--	Warm	5,000 (c)	1	2	--	--
H *	N.I.	--	--	Cool	1,000 (c)	30	52	--	--
I *	N.I.	--	--	Warm	300 (c)	<1	2	--	--
J	N.I.	Moist	--	27 ++	49 (d)	4	6	--	--
K	N.I.	Moist	--	27 ++	3.4 (d)	4	--	--	--
L	N.I.	--	--	--	4.6 (e)	<1	5	--	--
M	Sand	16	6.1	Warm	0.8 (f)	--	--	12	--

N.I. = No Information
* Organisms Added
+ Significant Industrial Component
# Soil Temperature
++ Sludge Temperature

**Units Reported:
(a) pfu/0.1 ml eluate
(b) pfu/g sludge-soil (dry wt)
(c) pfu/g soil
(d) pfu/g dried sludge
(e) $TCID_{50}$/g sludge-soil (dry wt)
(f) MPN CPU/g sludge-soil

extraneous contamination occurred is purely speculative. Unfortunately, unsludged fields which could have served as controls were not monitored.

The assignment of viral inactivation rates in sludge-amended soil rests largely on studies in which exogenous viruses were mixed with sludge and subsequently introduced into a soil system. It is important to note that during conventional wastewater treatment, viral removal occurs by adsorption to sites on or within the actively growing sludge floc. When viruses are mixed with non-metabolizing sludge, a majority of virions should become attached to the sludge; however, some viruses will remain free (i.e., not associated with the sludge). Thus, unless an effort is made to separate the fluid and solid phases, a portion of the seeded viruses will not be bound to the sludge particles.

Early work by Damgaard-Larsen and associates (1977) addressed the survival of a representative enteric virus in several soil types. Field scale lysimeters measuring 100 cm in diameter and 125 cm deep contained either a heavy clay soil (pH 7.4), a neutral sandy soil (pH 6.0), or an acidic sandy soil (pH 4.8). Anaerobically digested, dewatered sludge was seeded with Coxsackievirus B3, distributed on the surface of each lysimeter, and mixed into the soil with a spade. Viruses were measured by directly assaying the eluate of soil samples collected within the top 20 cm of soil. The experiment was started at the beginning of December, 1975, and sampling continued through May, 1976. Temperature and precipitation recorded over this test period were typical of the seasons observed in Denmark. While December temperature averaged 3.5°C, the months of January through March were much colder with average temperatures ranging from -0.3°C to 0.3°C. April and May warmed, with mean temperatures of 5.4°C and 10.6°C, respectively. February and March were the driest months with about 2 cm of precipitation per month, with a total of 30 cm recorded over the entire 6 month test period. The observed viral survival was quite similar across all soil types. As reflected by an initial T_{90} of about 30 days, viral inactivation was slightly faster in the sandy soils (Table 14, line codes A and C). In the clay soil, initial viral survival was more extended with T_{90}/T_{99} values of 56 and 100 days (Table 14, line code B). A more dramatic drop in recoverable viruses occurred late in the study as weather warmed in April and May, reaching maximal temperatures of 26°C. At the last sampling time, there was little difference in the number of viruses recovered from any soil type, reflecting at least a 4 $\log_{10}$ loss when compared to the initial viral levels present at the start of the study.

The survival of poliovirus 1 (LSc2ab) seeded into raw waste activated sludge applied to a vegetable garden plot was reported by Tierney and associates (1977). Two days after application the garden was hoed to mix in the applied sludge. Initial viral levels in sludge-amended soil were between 3×10^2 and 5×10^3 pfu/g as a result of this treatment. Soil samples were collected from the surface to a depth of 7.5 cm and eluted in a 50% suspension of antibiotic medium. This eluate was clarified by decanting through glass wool and filtering through a 0.45 µm cellulose acetate membrane filter. This procedure resulted in viral recoveries of 77% to 87% of input virus over four trials; however, no virus could be detected at levels below .1 pfu/g. During a winter test initiated in Cincinnati, Ohio, in 1973, poliovirus was isolated through 96 days of monitoring with an estimated T_{90} of 30 days and a T_{99} value of 52 days (Table 14, line code H). Inactivation was much more rapid during the early summer growing periods. In both 1974 and 1975, 90% of the poliovirus had been inactivated by 1 day with an additional one $\log_{10}$ loss by 2 days (Table 14, line codes I and G, respectively). In these summer tests virus persisted for only 8 to 11 days. Nearly 38 cm of precipitation and temperatures ranging from -14°C to 27°C were noted during the winter test. Temperatures during the 1974 and 1975 summer periods were very similar, ranging from about 20°C to 34°C. Rainfall measuring 13 cm was reported for the 1975 summer season. Thus, cooler temperatures favored poliovirus survival.

In conjunction with monitoring studies at the Fulton County sludge disposal site, researchers with the Metropolitan Sanitary District of Greater Chicago conducted experiments designed to evaluate the survival and movement of viruses in this agricultural soil (Lue-Hing et al, 1979; Metropolitan Sanitary District of Greater Chicago and IIT Research Institute, 1979). Poliovirus 1 or echovirus 7 were suspended in anaerobically digested sludge which was applied to the surface of prewetted soil columns (12 cm diameter x 31 cm length). Immediately after sludge addition, the contents of one column were completely mixed and assayed for viral concentration. Distilled water was added to each column at a rate of 100 ml/day for 5 days. At that time another column was dismantled, mixed, and analyzed for surviving viruses. Results of this testing showed that 85% of the poliovirus initially present had been inactivated after 5 days. Similarly, 81% of the echovirus was no longer recoverable. It should be noted, however, that while 70% of the input poliovirus could be recovered from the soil at day zero, only 8% of the echovirus was eluted from sludge-amended soil at the beginning of the experiment. These observations suggest an inherent procedural problem in evaluating the survival of echovirus 7 in soil.

To study the fate of representative enteric viruses in sludge-amended soil Bitton and associates (1984) seeded digested lagooned sludge with either poliovirus 1 (LSc) or echovirus 1 (Farouk). To ascertain the degree of viral association with solids, virus-sludge mixtures were centrifuged after the 60 minute mixing period and supernatants were assayed for infectious viruses. The percentage of solids-associated virus was then calculated from the number of viruses recovered from the whole sludge mixture and the number remaining in the liquid fraction following centrifugation. Notably, while over 95% of the poliovirus were no longer found free of the solids fraction, only 20% of the echovirus had become solids-associated. This virus-inoculated sludge was then applied as a liquid to a depth of 2.5 cm onto undisturbed soil cores (16 cm diameter x 33 cm length). The columns were wrapped with duct insulation and exposed to natural weather conditions prevailing in north central Florida. The applied sludge was allowed to drain on top of the soil for a period of up to 4 days during which time aliquots were removed and assayed for surviving viruses. Subsequently, the sludge was mixed with the top 2.5 cm of soil. Surface soil was monitored until viruses were no longer detected.

The first pair of columns was treated with sludge in the beginning of June, 1978. After 4 days of drying, 10% and 50% of the polioviruses observed after 1 day were still recovered in the sludge layer. After mixing into the soil, each column contained about 4 x 10^4 pfu/g of amended soil. Although virus was recovered for an additional 31 day period, the rate of inactivation was very rapid with a T_{90} of approximately 3 days and an estimated T_{99} of 7 days (Table 14, line code D). During this 30 day period, the surface soil moisture decreased from nearly 15% to 0.2%; however, rainfall totaling 14 cm did increase the soil moisture at intermediate times. As might be expected in exposed columns (i.e., not buried), soil temperature was largely constant with depth and ranged from 24°C to 29°C.

A similar series of experiments that included echovirus 1 was conducted beginning in October, 1978. Unfortunately, the monitoring scheme used did not provide timely samples to allow the calculation of die-off rates with much certainty. Specifically, between 5 and 18 days after the sludge had been mixed into the soil, no samples were taken and by day 18 neither virus could be recovered from the surface soil. However, by graphical estimation a T_{90} value of 2 days for poliovirus and 7 days for echovirus appears reasonable (Table 14, line codes F and E, respectively). During the 3 weeks in which virus survival in sludge was followed, soil temperature ranged from 18°C to 27°C and only .13 cm of rain was recorded. Soil moisture decreased from 7.5% to 1% reflecting a steadily drying environment. Overall, little difference was observed in the rate of viral inactivation in either the summer or fall tests, although echovirus appeared to die off somewhat slower. It should be noted, however, that relatively little difference existed in environmental conditions during these test series.

Parasites

In terms of extended survival, parasites have imposed the greatest microbial constraint in the application of waste products to land. Until recently, however, few studies have examined the fate of this group of microorganisms in sludge-amended soil.

An early report by Köser (1967) noted the recovery of about 12 *Ascaris* eggs, 0.7 worm eggs, and 3.5 *Trichuria* eggs per liter of digested sludge destined for land application in the Federal Republic of Germany. At 2, 3, and 5 weeks after sludge application, 1.1 *Ascaris* ova were still detected in 1000 g of soil. Also working in the Federal Republic of Germany, Forstner (1968) placed bags containing *Ascaris* (10^5) and *Taenia* (10^4) eggs in fields treated with digested sludge. Testing at 2.5, 3.5, and 5 months after application demonstrated intact eggs from all samples. Eggs were infectious for mice only through 3.5 months. Using virtually the same approach many years later, Strauch and associates (1981) seeded *Ascaris suum* eggs into silk bags that were layered within sludge applied to several different forest stands in the Federal Republic of Germany. Samples were removed, resuspended in water, incubated for 40 days at 29°C, and viewed for larval stages. Positive samples were administered orally to mice. Two days later liver sections were scored for the presence of larval III stages. The viability of *Ascaris* ova was quite similar regardless of the type of soil, forest stand, or season of sludge application. Eighty-four days after summer sludge application, ova were no longer infectious at any of eleven test sites. Eggs were not infectious after 78 days in ten plots receiving sludge in the winter; however, they could be recovered until 107 days in a single test plot. Notably, in excess of 10^6 eggs were present in each sample at the beginning of each test series.

A single report in the available Russian literature details the survival of ascarid eggs in the soil of sewage farms and in sewage deposits on ooze grounds (Drozdova et al, 1972). For purposes of this review, data reflecting ova survival at the sewage farm will be included, recognizing that by strict interpretation the similarity with sludges generated during biological waste treatment may be limited. In one series of experiments pits were excavated in the soil, a 5 to 8 cm thick layer of sewage deposits was introduced, and soil was then filled in over the deposits. Within these sewage deposits created at various depths (from 10 to 60 cm), ascarid contaminated soil (200 to 300 eggs/g) held in containers of iron screen or aluminum hair curlers was implanted. Another experiment involved introducing eggs into existing ooze grounds by attaching sample holders at various depths on 1.5 meter long iron bars, which were subsequently driven into the ground and retrieved at various sampling times. In farm soils with sewage deposits added, eggs survived up to 360 days at depths of 10 and 20 cm and up to 540 days at a depth of 60 cm. In sewage deposits on ooze grounds, ascarid eggs lost viability much more rapidly with only a 30 day survival period at depths between 5 and 40 cm. Because of the dry environmental conditions in the Volgograd Region of the Soviet Union, eggs nearest the soil surface had the shortest survival times. (Indeed, when placed directly on the soil surface, eggs survived for no more than 25 days.) The authors speculated that increasing organic matter and microbial load intensified biochemical processes acounting for the more rapid egg extinction in soils amended with sewage deposits.

In a Norwegian study designed to follow the survival of parasite eggs in sludge-amended soil, Bergstrom and Langeland (1981) seeded pig feces containing *Ascaris suum* ova and sewage sludge from a mechanical-chemical treatment plant into a humus soil. After thorough mixing, the amended soil in the test plots contained 2,000 *Ascaris* eggs per gram. Seven of ten test plots were subsequently planted with either radishes, turnips, or lettuce and regularly watered. During a second and third growing season grass was planted on all ten plots. Samples were analyzed over a 27 month period. Based on morphology, eggs were scored as dead/alive and infectious/noninfectious. Undamaged eggs (either embryonated or unembryonated) were reported as infectious. The authors noted that the concentrations of total ova in the top 6 cm of soil decreased over the first summer growing season (15 wks) with the most dramatic loss in the unplanted plots. Concomitantly, however, increasing numbers of *Ascaris* eggs were recovered from soil depths of 6 to 25 cm suggesting displacement through the soil profile rather than destruction of ova in the surface soil. Embryonation of the eggs was maximal (65 to 80% of total egg count) at 7 to 10 weeks on the soil surface (0 to 3 cm) and at 15 weeks (90% of total egg count) between 3 and 25 cm depths. Even after 27 months, 50% of the total eggs observed at 3 to 25 cm depths were judged infectious, i.e., approximately 1,000 ova/g of soil were embryonated. Far fewer *Ascaris* eggs at the soil surface were infectious by the end of the study with approximately 125 ova/g (25% of 500 total count) and 2 ova/g (5% of 50 total count) in the planted and fallow plots respectively. The authors concluded that this greater egg destruction near the soil surface could be attributed to solar radiation, high temperature, and periodic drying.

Several reports have described efforts to ascertain the survival of parasitic ova in various regions in Canada. Graham (1981) detailed studies undertaken in Ontario to evaluate the survival of parasite ova in sludge applied to agricultural soils. Initial screening was conducted at farm A where the last sludge application occurred approximately 1.5 years before sampling. A second farm B receiving sludge in the time frame of sampling was also included in the monitoring program. *Ascaris* ova were recovered from dried sludge obtained from a storage pit and from a soil core sample at farm A, but none of the isolates was viable. No parasite recoveries were made from surface soil samples. At farm B no ova were detected in surface samples collected from a field that had received sludge 8 to 10 months previously. In a pasture receiving sludge, ova were recovered on the day of application. Two days later *Ascaris* and *Toxocara* ova were recovered from a sludge sample on the soil. Several weeks later, only one of four samples yielded ova.

To establish a better time frame of parasite survival, experimental plots of fine sandy loam were treated with digested sludge seeded with ova of *Ascaris suum*, *Toxocara*, or *Taenia*. Applications were made at approximately monthly intervals beginning in June, 1977. Sludge was applied to the surface of pastureland (with grass cover), bare soil, and bare soil that was subsequently tilled to a depth of 2 to 3 inches after sludge application. Both surface and subsurface samples as well as forage from the pasture were analyzed for ova. Despite the fact that sludge was seeded with parasitic eggs, levels of *Ascaris* eggs in sludge residue on the surface of bare soil were ≤ 1 per gram even within 1 to 9 days following applications in both June and July. The mean air temperature was highest at this time, reaching 29°C in June and July, and a mean soil temperature of 47°C was recorded in June. The recovery of *Ascaris* ova during sampling following the August sludge application allowed the establishment of a T_{90} value of 17 days with eggs recovered through 21 days (Table 15, line code C). After the spreading of sludge in September onto bare soil, no loss of ova was documented through 22 days and follow-up samples delayed until 284 days later yielded no *Ascaris* ova. Ambient temperatures were only slightly cooler in September, reaching a mean of 18°C; however, recorded soil temperatures (13°C) were 10°C lower than in the month of August.

The pattern of *Ascaris* ova recovery from vegetation and soil was similar following sludge application to pastureland in both July and August. Initial dieaway with estimated T_{90} of 62 days and 74 days was observed for July and August, respectively (Table 15, line codes A and B). Unlike results obtained in the bare fields, however, viable ova were continuously recovered at very low concentrations (0.1 ova/g) through 140 to 150 days. When pastures were sludged in September, once again T_{90}/T_{99} values of 65 and 75 days were estimated from available data (Table 15, line code D). However, *Ascaris* ova were recovered through 539 days on this test plot with active larvae last noted in samples collected 139 days after sludge application. Sludge contaminated with *Toxocara* ova were also spread on pastureland in September. Their survival largely paralleled that of *Ascaris* with T_{90}/T_{99} values of 77 days and 84 days estimated (Table 15, line code E). *Toxocara* ova with larvae

TABLE 15. SUMMARY OF SURVIVAL OF PARASITES IN SLUDGE APPLIED TO SOIL

PART I.

Line Code	Reference	Sludge Type	% Solids	Sludge Application	
				Rate (t/ha)	Mode
A *	Graham, 1981	Digested (Anaerobic)	--	--	Spread
B *	Graham, 1981	Digested (Anaerobic)	--	--	Spread
C *	Graham, 1981	Digested (Anaerobic)	--	--	Spread
D *	Graham, 1981	Digested (Anaerobic)	--	--	Spread
E *	Graham, 1981	Digested (Anaerobic)	--	--	Spread
F *	Jakubowski, 1984 +	Digested (Anaerobic)	4	10	Sprayed
G *	Jakubowski, 1984 +	Digested (Anaerobic)	4	9	Sprayed
H *	Jakubowski, 1984 +	Digested (Anaerobic)	4	9	Sprayed, tilled
I *	Jakubowski, 1984 +	Digested (Anaerobic)	4	9	Sprayed
J *	Jakubowski, 1984 +	Digested (Anaerobic)	4	9	Sprayed, tilled
K	Lehmann, et al, 1983	Digested (Anaerobic), Lagooned	--	--	Spread, tilled

PART II.

Line Code	Soil Characteristics			Mean Ambient Temp. (°C)	Initial Microbe Conc. **	Die-off			
						Surface (0-5 cm)		Depth (5-15 cm)	
	Type	Moisture (%)	pH			T_{90}(d)	T_{99}(d)	T_{90}(d)	T_{99}(d)
A *	Fine Sandy Loam	5-22	--	26	4 (a)	62	68	--	--
B *	Fine Sandy Loam	10-22	--	18	23 (a)	74	81	--	--
C *	Fine Sandy Loam	11	--	18	8 (b)	17	--	--	--
D *	Fine Sandy Loam	10-22	--	10	16 (a)	65	75	--	--
E *	Fine Sandy Loam	10-22	--	10	22 (a)	77	84	--	--
F *	Clay	22	5-6	22	180 (b)	30	--	--	--
G *	Clay and Silt	22	6.3-7.7	12	20 (b)	90	--	--	--
H *	Clay and Silt	17	6.3-7.7	25	15 (b)	200	--	--	--
I *	Sandy Loam	12	6.8-7.9	9	27 (b)	80	--	--	--
J *	Sandy Loam	15	6.8-7.9	25	8 (b)	200	--	--	--
K	N.I.	--	--	--	--	270	500	--	--

N.I. = No Information
* Organisms Added
+ Personal Communication

** Units Reported: (a) ova/g of soil, sludge and vegetation mixture
(b) ova/g sludged soil

were recovered even after 558 days. The maximum air temperature means recorded for September and October were 18°C and 13°C, with temperatures becoming dramatically colder in November. Snow covered the test site from December through March. While *Taenia* ova were applied to pastureland as well, inefficient recovery methods hampered data interpretation. These ova were recovered 9 days but not 16 and 22 days following sludge application in July, and at 26 and 34 days, following the August sludge application.

The only results presented from tilled plots reflected no *Ascaris* loss in the 0 to 2 cm depth over 21 days following either August or September application. After mixing with the soil, however, fewer ova were recovered in analyzed samples.

Results of a second Canadian-based study conducted in fields receiving lagooned sludge near Calgary were reported by both Lehmann (1983) and Wallis (1984) along with their respective co-workers. At this site researchers evaluated the survival of naturally occurring parasitic ova. Looking first at fields sludged over a 3 year period beginning in 1978, Lehmann and associates noted that while a larger portion of parasite eggs were found at greater depths with time after sludging, more than 68% of those recovered were found in the top 3 cm of the soil profile. Since these fields had been disced in the course of normal agricultural operations, it is likely that eggs displaced deeper in the soil would have survived longer. Perhaps more importantly, ova were not randomly distributed on the fields when a large number of samples were collected and processed in parallel, showing instead a trend toward horizontal clumping. Increased sample size did not alleviate this uneven distribution. Thus, while a graphical plot of egg survival on these sludged fields after data transformation by natural logarithm showed a predictive T_{90}/T_{99} of 270 days and 500 days (Table 15, line code K), respectively, pockets containing high numbers of surviving ova may exist. In addition, no control field data were analyzed and the number of ova contributed by domesticated or wild animals could not be ascertained.

In May, 1981, digested, lagooned sludge undergoing surface application was analyzed and found to contain over 11,000 eggs/kg dry weight (Lehmann et al, 1983). Organisms identified included *Ascaris* sp., *Enterobius vermicularis*, *Toxocara canis*, *Toxocara cati*, *Toxascaris* sp., and *Trichuris* sp. Samples of sludge taken 1 week later from the surface of a field yielded over 7,000 eggs/kg. The field was then disced and allowed to lie fallow. In August no viable eggs were found in 15 soil samples. Soil moisture averaged 3.8% in the top 2 cm of the field at that time. Dry conditions undoubtedly hastened ova destruction. Similarly, rapid loss of ova was observed in the summer of 1982 when sludge containing 11,000 ova/kg was spread on grassland. Seven days after application, 7,000 parasite eggs/kg were recovered. Twenty-eight days after sludging, no eggs were found. Virtually identical results were obtained when the same sludge was injected and disced 1 week later (Wallis et al, 1984). Virtually no ova were recovered within 4 weeks from the injected field.

The results observed on control fields for this Calgary monitoring program highlight the contribution of both domestic and wild animals to the occurrence of parasitic ova in soil. Parasite eggs were recovered from unsludged, grazed fields (located approximately 16 km from the sludging operation) at levels ranging from about 200 to 900 ova/kg of soil. Examination of manure samples in the field resulted in values as high as 91,000 eggs/kg confirming that cattle contributed to these background levels. From spring through fall, parasite eggs, presumably shed by wild deer, were found on the control hayfield reaching levels of 500 eggs/kg. A spot check of deer droppings yielded over 2,200 eggs/kg.

Surprisingly little work has been published addressing the survival of parasitic ova in sludge-amended soils in the United States. A nationwide survey for helminthic ova at nine selected sludge disposal sites was completed by Theis and associates (1978). Sludge application had been practiced for a minimum of 5 years at each site. Generally, four to six sludge samples and 10 to 16 soil samples were examined at each site. Ova were observed in sludges at only four of the nine sites. Furthermore, parasitic ova were recovered from 8% and 13% of the soils at two of these four locations. Very few of these positive samples had more than 50 eggs/g of soil. The most frequently isolated ova were those of Ascaris lumbricoides and Toxocara sp. Monitoring at the Oakland-East Bay soil enrichment program suggested a correlation between sludge loading and positive ova recoveries (Theis et al, 1978). In examining 124 soil samples from plots receiving high rate loading over a 4 year period, 21% yielded parasitic ova. Analysis of 120 samples from plots receiving an order of magnitude lower loading in the same time frame resulted in only 12% positive. Notably, unsludged control fields also had 8% of 75 samples positive for helminth eggs in this survey.

Yanko and associates (1978) reported the recovery of indigenous Ascaris ova in sludge-amended soils as part of the Los Angeles/Orange County Metropolitan Study conducted in California. Total ova were concentrated using zinc sulfate flotation and their viability was scored using a Nile Blue sulfate stain. Viable Ascaris ova were detected in all sludged fields following the January, 1977 application of 90 t/ha and in June, 1977 at the time of crop harvest. In all but one case, less than 1 ovum/g of sludged soil was reported, and viable ova were detected at levels of 0.2 ovum/g or less. Levels of viable ova were basically the same in January and June samples. Notably, Ascaris ova were regularly detected in unsludged fields as well; however, these eggs were not scored as viable and levels were 3- to 10-fold lower than those observed in test fields.

Preliminary unadjusted data on the survival of Ascaris ova in soils of three geographical locations within the continental United States have been provided from the U.S. Environmental Protection Agency (Walter Jakubowski, personal communication, 1984). Ascaris suum ova were collected and concentrated from the feces of infected pigs. To duplicate actual treatment practice, these eggs were seeded into laboratory scale anaerobic digesters operated at 35°C. After 2 weeks this digested sludge was mixed with unseeded municipal anaerobic sludge prior to land application. Study sites were located in New Orleans, La., Lubbock, Tx., and Cincinnati, Oh., and included both tilled and grass covered plots. Sludge was sprayed onto the soil surface and, as appropriate, tilled to mix the sludge into the topsoil. Application was done in January, 1984, at the New Orleans and Lubbock sites and in June, 1984, at the Cincinnati site. Soil samples on grass plots were collected at a 0 to 10 cm depth while tilled plots were sampled at 10 to 20 cm as well. The location of samples within each test plot had been determined prior to initiation of the experiment using a randomized subplot sampling protocol. An assortment of environmental parameters including rainfall, temperature, solar radiation, and soil pH and moisture were recorded at each site.

The most rapid loss of Ascaris ova was observed when sludge was applied to an untilled plot in Cincinnati with an estimated T_{90} of about 30 days (Table 15, line code F). Conversely, through the November sampling period, no significant loss of ova was observed in the tilled plot in the top 10 cm of this predominantly clay soil. No cover crop had been planted on the field. Air temperatures ranged between lows of 8°C and highs of 31°C from June through October. For the month of November temperatures were decidedly cooler with recordings of below 0°C to 15°C. Soil moisture measured 5 cm beneath the surface in the tilled plot generally ranged from 15% to 32% (probe detection limit) during the months of the monitoring program.

Winter application of Ascaris laden sludges in New Orleans and Lubbock resulted in significantly longer survival times. Despite an increase in numbers of reported ova in fall samples collected at the New Orleans site, an initial T_{90} value of about 90 days was observed in the untilled plot (Table 15, line code G). Ascaris survival in the tilled plot planted with cotton allowed the estimation of a T_{90} of 200 days (Table 15, line code H). However, relatively high numbers of ova were still being recovered from all plots in December. Air temperatures ranged from a low of 1°C to 20°C from January to March and from 10°C to 35°C from April to August. Soil moisture was cyclic during the year at the 5 cm depth of the tilled plot in New Orleans. During January and February, 30% soil moisture values were reported, dropping gradually to a consistently low 5% moisture during April and May. Through August soil moistures fluctuated between 10% and 30%, reflecting increased rainfall (15 to 20 cm per month) during the summer.

The pattern of Ascaris ova survival was similar at the Lubbock site where relatively rapid dieaway on untilled plots was observed with an apparent T_{90} of about 80 days (Table 15, line code I). The loss of eggs in the tilled plot also planted with cotton occurred with an estimated T_{90} of 200 days in the top 10 cm of soil (Table 15, line code J). Notably, ova survived much better in the tilled field at the 10 to 20 cm depth with almost 50% of the initial levels recovered in November sampling. Weekly average air temperatures at this site ranged from a low of -9°C to 20°C in January through March, from 3°C to 37°C in April through September, dropping to near freezing lows by late November. Soil moisture in the sandy loam soil at a 5 cm depth in the tilled plot was noticeably lower than at the New Orleans site. From January into March, soil moisture values of less than 10% were recorded. The use of irrigation water beginning in the spring coupled with the occurrence of sporadic rainfall in the summer and fall months (2 to 12 cm/month) resulted in a cyclic pattern of soil moisture ranging generally from 10% to near 25%. Notably, drying was very rapid with peak soil moistures never consistently maintained.

Embryonated Ascaris eggs were observed at all sites. However, at the New Orleans and Lubbock sites such forms were not recovered until April. After the summer sludge application in Cincinnati about one-half of the eggs in the grass plot had embryonated by 3 weeks and more than 90% of the ova in the tilled plot had embryonated within 2 months. Presumably, cooler weather delayed the developmental progress of the ova in sludge applied during the winter months. Infectivity tests showed that all sites contained Ascaris eggs capable of infecting mice.

In an extensive study conducted in Illinois, Fitzgerald (1981) looked at another aspect of Ascaris survival on sludged soil by evaluating the transmission of parasitic ova to pathogen-free pigs after their exposure to various types of digested sludge. Sludges applied to pens contained indigenous parasites (about 50 ova/L) and, in some cases, exogenously added Ascaris suum ova (about 100/L). Sixteen to 18 weeks after the beginning of exposure, pigs were sacrificed and necropsied. In one experiment, 14 of 16 pigs exposed to ascarid ova seeded into sludge or water applied to soil became infected, while only 1 of 16 pigs became infested as a result of exposure to digested, stored sludge. Exposure of these animals began 7 months after the last sludge application in October. Seven of 11 pigs exposed to freshly digested sludge 1 week after its application became infested with ascarids. Interestingly, no parasites other than Ascaris sp. were ever observed in animal tissue, even though sludge samples contained indigenous ova of both Trichuris and Toxocara sp.

In a supplemental study, Fitzgerald (1981) observed some embryonation of seeded Ascaris ova in sludged soil as early as 1 to 2 weeks after application. However, at 7 weeks after sludging an average of 62% of the recovered ova were embryonated and presumably infectious. Over this 7 week monitoring period, no substantial loss of total Ascaris ova was noted. Thus the potential for transmission of Ascaris from sludge to animals exists. Although infestations were relatively light, the establishment of ascariasis within a herd could occur as infected animals multiplied the number of ova present in the environment.

TRANSPORT OF MICROORGANISMS FROM SLUDGE-AMENDED SOILS

Another important aspect of sludge application on land is the potential dispersion of pathogens in the environment. Sludge-associated organisms could conceivably be carried off-site in runoff (i.e., horizontal transport) or by movement through the soil profile largely as a result of water percolation (vertical transport). The degree to which either type of transport occurs has been addressed in selected studies, although few investigations have been specifically designed to evaluate the movement of pathogens.

Commonly, monitoring schemes designed to address horizontal transport include interception of runoff within or at the perimeter of a sludged site. Alternatively, surface waters potentially influenced by runoff from the sludge-amended area can be sampled. While the latter approach is intended to directly assess the impact of horizontal transport, the data are frequently difficult to interpret when, for example, dilution factors are great or other sources of pollution exist.

Vertical transport can be measured by organism recovery from soil or water with increasing depth. Since contamination of groundwater is an overriding concern, however, the majority of studies have used groundwater monitoring approaches. For most microorganisms a much larger water sample can be analyzed when compared to soils. However, if microbes are transported vertically through the soil profile, such movement need not be a continuous process. Rather, sporadic transport events associated with unique environmental conditions are likely to occur. Since groundwater monitoring is not a continuous process, the investigator would have to be in the right place at the right time looking for the right organism to record a positive measurement. Thus, multiple approaches should be included in any studies designed to address microbial transport from sludge-amended soils.

Bacteria

Data on either horizontal or vertical transport of bacterial pathogens at operational field sites are lacking. This can be attributed to the relatively low levels of these organisms present in applied sludges.

Laboratory studies conducted by the municipality of Metropolitan Seattle were designed to observe the transport of selected bacterial pathogens as well as indicator bacteria through soil from the nearby Pilchuck Tree Farm (Andrew Heyward, personal communication, 1983). In 1982, soil cores measuring approximately 61 cm in diameter by 46 cm deep were collected and moved into the laboratory. Approximately 2.5 cm of dewatered sludge was uniformly layered on top of three cores, avoiding the edges of the cores. Two additional unsludged cores served as controls. Over a 48 hour period, about 15 cm of deionized water was applied to each core. Soil was then allowed to dry out (5 to 6 days) before another 15 cm of water was applied. In all, three such cycles were completed, with percolate waters being collected and analyzed with each water addition. Bacterial analyses using appropriate enrichment and MPN procedures included those for *Shigella*, *Salmonella*, *Yersinia*, total and fecal coliforms, and fecal streptococci. No *Shigella* sp. were detected in either applied sludge or percolate water. Indigenous *Salmonellae* were detected in only one sample; the sludge on top of a core after the completion of the experiment yielded 70 *Salmonella*/100 g of sludge. *Yersinia* sp. were detected in dewatered sludge applied to the cores at a level of 2.4×10^3/100 g and in sludge at the end of the test at a concentration of 1.4×10^4/100 g. These bacteria were detected in percolate water after both the first and third cycle of "rainfall" at levels ranging from 4 to 61 *Yersinia*/100 ml in two of the three test cores. No *Yersinia* were recovered from either of the control core percolates. Similarly, fecal coliforms and fecal streptococci were recovered from all test core percolates with the largest numbers appearing in samples collected after the initial water addition. However, maximal levels of bacteria observed represented 0.1% of the fecal coliforms in applied sludge and 0.01% of the fecal streptococci. Neither fecal coliforms nor fecal streptococci were observed in percolates of control cores. Total coliforms were recovered from both test and control core percolates through all three cycles. However, total coliform levels in the percolates of sludged cores were generally 10^2- to 10^3-fold higher than control core percolates. Thus, while bacterial levels were substantially reduced by migration through 46 cm of soil, the application of high rainfall equivalency volumes (1 year's rainfall within 1 month's time) resulted in limited bacterial transport in sludge-amended soil.

Van Donsel and Larkin (1977) noted that about 1% of the total Mycobacterium bovis sprayed onto vegetable plots constructed in wooden boxes with plastic underliners were recovered in percolate tanks. However, because of the nature of the test plots it was possible for surface water to drain around the sides of the plots into the tank. Other than these reports, virtually all studies of bacterial transport have used either traditional or tentative indicator organisms.

Two studies have looked at the bacterial quality of runoff from sludged fields. One of these, reported by Dunigan and Dick (1980), involved the surface application of sewage sludge to relatively impermeable soil located at the Agronomy Farm of Louisiana State University at Baton Rouge. Field slopes varied from 3% to 5% (Dick, 1977). Surface water runoff was intercepted at the base of each plot in a polyethylene barrel. While the emphasis of this research was nutrient loss, fecal coliforms were analyzed by MPN procedures in two experiments. Samples were collected after each rainfall until values from sludged plots reached those from control plots. In March, 1976, 15 t/ha were applied to the surface over an existing ryegrass cover. Elevated levels of fecal coliforms at about 1.3 x 10^6/100 ml were observed consistently in runoff water collected at 7, 9, and 11 days after sludging. Bacterial levels from parallel control plots were about 2.0 x 10^3 fecal coliforms/100 ml. After a 25 day dry period, fecal coliforms in runoff from sludged areas were substantially reduced to near background levels. A second experimental series was initiated in July, 1976, with loadings of about 16 and 29 t/ha on separate fields planted with pearl millet. Somewhat higher fecal coliform levels (5.5 x 10^6/100 ml) were observed in runoff water from the 16 t/ha plot intercepted 13 days after sludge application. After 15 days another rainfall event released 2.2 x 10^6 fecal coliforms/100 ml from the 29 t/ha plot but only 8 x 10^5/100 ml from the 16 t/ha field. Bacterial levels in runoff continued to decrease, especially after a 3 week dry period. Forty-two days after sludge application, indicator bacterial levels were comparable in surface runoff from both sludged and control plots. Thus surviving bacteria were present in runoff from sludged fields with numbers increasing as sludge loading increased.

Monitoring at the Fulton County land reclamation site involved sampling surface waters including reservoirs and a stream flowing through the site (O'Brien et al, 1978; Metropolitan Sanitary District of Greater Chicago and IIT Research Institute, 1979; Lue-Hing et al, 1980). Indicator bacteria including standard plate count, total coliforms, fecal coliforms, and fecal streptococci were analyzed using standard membrane filtration procedures. Lakes receiving runoff from either sludged or unsludged areas showed indistinguishable bacterial profiles over a 6 year monitoring period when four to 15 samples were analyzed yearly. A third reservoir draining a sludge application area and apparently downslope from sludge holding basins likewise had similar levels of coliforms and fecal streptococci. Notably, standard plate count increased through 1975, suggesting some runoff may have occurred. At this site from 20 to 98 samples were analyzed each year. Water samples from Big Creek were taken as it entered and exited the reclamation site.

With few exceptions bacterial levels were lower at the downstream sampling station. Interpretation of stream data is complicated by the fact that the Canton Sewage Treatment Plant located about 20 km upstream from the first sampling station introduced significant quantities of indicator bacteria into Big Creek, thereby elevating the background against which the Fulton County site was evaluated. Monthly groundwater monitoring was also conducted at three wells located outside the sludge application sphere of influence and at two wells located within sludge impacted zones. From 1971 through 1977, levels of indicator bacteria were virtually identical in all wells. The depth to groundwater, however, was not reported.

Working in Washington state, Edmonds (1976) concluded that there was no consistent evidence of vertical fecal coliform transport as measured in water from an 8 m well or springs draining sludged areas. Occasionally, coliform bacteria were observed in samples collected from tension lysimeters at a 180 cm depth beneath the sludged site. Maximal levels of 470 total coliforms and 160 fecal coliforms/100 ml were reported. The reliability of tension (ceramic) lysimeters is questionable, since microorganisms can be either retained within the ceramic matrix if ionic conditions are favorable or grown within the lysimeter if nutrients are available.

At a sludge application site near Fairbanks, Alaska, Johnson (1979) monitored fecal coliforms in soil at depths of 0.3, 0.9 and 1.5 m in fields receiving aerobically digested, dewatered sludge. Coliform bacteria were found only near the surface of these rototilled fields. No positive recoveries were made at either the 0.9 or 1.5 m depths. However, it should be noted that over the time period of the study, the total recorded rainfall was 9.7 cm and 3 cm on these test plots (sludge treatments beginning in July and August, respectively), with the heaviest single rainfall being 1.7 cm over a 24 hour period. This low amount of precipitation coupled with a soil permeability ranging from only .5 to 1.5 cm per hour, would have provided little water movement through the soil profile.

Hagedorn (1980) also monitored groundwater using point wells installed to a depth of 100 cm beneath fields treated with anaerobically digested sludge. Four different sites representing various soil types were included in this study. Groundwater monitoring was not possible during a summer test series as water tables were too low. However, fecal coliforms were detected in wells on the Dayton (fine clay) and Amity (fine silt) plots during the first 4 weeks following sludge application at levels rarely exceeding 200/100 ml. By the fifth week of sampling, numbers at both sites were $<2 \times 10^1$/100 ml where they remained throughout the sampling period.

As part of a study conducted in Oklahoma during 1981, Ibiebele and Inyang (1986) sampled soils amended with undigested sludge at various depths to 30 cm. Three test plots (4.8 x 9.6 m) received increasing volumes of 760, 1,520, and 2,280 liters of sludge each while a fourth plot served as an unsludged control. Leachates were intercepted at a depth of 60 to 80 cm using a perforated polyvinyl chloride pipe with a capped bottom. Water sampled were collected using a peristaltic pump. Fecal streptococci and fecal coliforms were enumerated by standard membrane filtration procedures applied to water and to the settled supernatant of 10% soil suspensions.

These authors interpreted their data as showing fecal coliform transport to soil depths of 15 and 30 cm by 90 days after sludge application. However, in the majority of soil samples, the levels of indicator bacteria in the control fields were comparable to those in the sludged fields at all depths. Furthermore, the levels of indicator bacteria in the control plot leachate were generally equal to or greater than those observed in the water collected from sludged plots.

Field lysimeters (30 cm diameter x 1.8 m depth) were packed with two soil types (loamy sand and silt loam) to reflect appropriate soil horizons and subsequently treated with anaerobically digested, chemical sewage sludges (Liu, 1982). Lysimeters were exposed to prevailing environmental conditions in Ontario, Canada. However, supplemental irrigation was used to maintain soil moisture at one-third field capacity or greater (equivalent water was added to all lysimeters). Using membrane filtration techniques, no indicator bacteria were ever detected in leachates. Notably, exceedingly low levels of bacteria were reportedly present in the applied sludges (i.e., 10^5 total coliforms, 10^2 to 10^3 fecal coliforms, and 10^2 fecal streptococci per gram).

Two studies have detailed the behavior of clostridial spores in soil columns. Bourillet and associates (1981) filled laboratory columns (25 cm diameter x 30 cm length) with sterilized sand to a depth of 20 cm. Liquid sludge (6.6% solids) was applied to the surface to approximate a loading of 100 m^3/ha. Testing was initiated in a greenhouse setting and moved outdoors after frost conditions subsided. Over the month prior to outdoor exposure, 250 ml of sterilized water was added on five occasions. Once buried outside to a depth of 20 cm, the columns received only incidental rainfall. Control columns remained free of *Clostridia*. Beginning at 4 weeks and continuing through 12 weeks, only 1% to 6% of the total number of spores detected were recovered from sand beneath 6 cm. No bacterial spores were detected in column percolates until 8 weeks after sludge application. Even after 12 weeks only 0.02% of the clostridial spores were recovered in column filtrates.

In another effort to address *Clostridia* movement in soils, St. John and Matches (1982) placed heat-sterilized soil into small columns (2.5 cm diameter x 6 cm length). One gram of dewatered sludge was added to the top of the "semi-packed" soil columns. Sterile water was added dropwise for 24 hours (equivalent to 170 cm of rain), columns were dismantled, and the soil was analyzed at various depths. A gradient of *C. perfringens* was recovered, decreasing substantially with distance from the soil surface. Approximately 0.08% of the *Clostridia* found in the top 2 cm was detected below 6 cm in a glass wool plug with less than 15% recovered from the 2 to 4 cm depth. These observatious support those of Bourillet and co-workers (1981), suggesting very limited movement of *C. perfringens*.

Finally, results reported from a sludge injection site in Winnipeg, Manitoba, Canada, documented both horizontal and vertical movement of indicator bacteria away from the injection trench (Carroll and Ross, 1983). Samples collected 15 cm to the side of the sludge injection line frequently yielded MPN values as much as 10% of those recovered within the entrenched sludge. Similarly, vertical samples collected at a 45 cm depth (30 cm below the theoretical injection depth) frequently contained significant levels of total and fecal coliforms within 1 week of sludge application. However, these values did not increase as sampling continued at 1, 3, and 12 months later even though large numbers of indicator bacteria remained in the sludge pocket. These observations suggest that the initial organism movement was probably due to the injection process. Further, one could envision a "wicking" phenomenom carrying bacteria in the liquid phase into drier areas in the adjacent soil coincidental with sludge injection.

Viruses

The potential for direct viral pollution of surface water due to the application of sludges has been addressed in only two reports. Tierney and co-workers (1977) intercepted drainage water from vegetable plots that had been amended by the incorporation of poliovirus-inoculated, waste-activated sludge. As much as 1% of the added virus was recovered in drainage within 1 day of sludge application. The authors noted that during heavy rains surface soil was resuspended and carried into the collection reservoir. While these observations highlight the potential problem of horizontal viral transport, it should be noted that the construction of the experimental plots with plastic underliner may have compounded surface runoff since normal water percolation through the soil profile was impeded.

As part of the overall monitoring program conducted by the Metropolitan Sanitary District of Greater Chicago at the Fulton County land reclamation site, direct runoff from a field receiving sludge application was intercepted in a buried trough during two rainfall events in the spring and fall of 1976 (Lue-Hing et al, 1979). No confirmed viruses were reported from either a 4 L or 13 L sample. Companion monitoring of the effluent from the field's runoff capture basin likewise yielded no viruses during a 2 month study period. Sample volumes ranged from 2 to 8 L.

Viral monitoring of surface waters was also conducted over a period beginning in April, 1972, and continuing through August, 1979, at the Fulton County, Illinois sludge utilization site (Bertucci, 1981). Two sampling stations were established on Big Creek; one station was immediately upstream of the sludge application site while another station was downstream where Big Creek exited the site. It should be noted that discharges from the city of Canton's sewage treatment plant entered Big Creek upstream of the first station. A third station was located at the discharge of Evelyn Reservoir, which received both direct and indirect discharges from field runoff basins. Initially 4 L samples of creek water were concentrated for viruses; however, beginning in summer of 1976, volumes at all sites were increased to 20 L. Although polyelectrolyte 60 sandwiches were used to concentrate 20 L reservoir samples until November, 1975, all stream samples and subsequent reservoir samples were handled by a modified aluminum hydroxide precipitation method. Prior to November, 1975, primary rhesus monkey kidney cells were used to isolate indigenous viruses; thereafter, BGM cells were used. Plaque confirmation procedures were instituted after September, 1975. Generally, monthly sampling was continued through September, 1977, after which sampling was conducted on a quarterly basis. Prior to confirmation of lesions within the cell monolayer as viral plaques, pfu were reported in virtually every surface water sample with reported concentrations of 20 pfu/L not uncommon. However, once plaque confirmation procedures were followed, few samples yielded reportable viruses. Excluding a single instance in which poliovirus 1 was apparently introduced by laboratory contamination, only 4 of 94 surface water samples were positive for viruses. Of these, no positive samples were collected from the Evelyn Reservoir and only two positive samples were reported from both the upstream and downstream stations on Big Creek. Thus, there was no evidence of viruses reaching surface waters solely as a result of digested sludge application at the Fulton County, Illinois site.

To evaluate the possibility of viruses in sludge migrating through the soil profile at the Fulton County reclamation project, groundwater samples (20 to 40 L each) were analyzed approximately monthly for 1 year beginning in October, 1975 (Lue-Hing et al, 1979; Metropolitan Sanitary District of Greater Chicago and IIT Research Institute, 1979). Viruses were not recovered from either of two wells located within the sludge application area or from a control well placed in an area not receiving sludge.

Groundwater monitoring in sludge application areas has also been undertaken in Florida. Monitoring groundwater beneath a sludge disposal site in St. Petersburg, Wellings and associates (1978) recovered poliovirus type 2 on two occasions: first from an 8.5 m deep well 18 months after initiation of sludge disposal at the site and again from an 18 m well 6 months later. In a year-long study conducted in north central Florida, four wells located at a site where digested, lagooned sludge was applied to fields were monitored for viruses bimonthly (Farrah et al, 1981). Total volumes concentrated ranged from 1,100 to 2,650 L with no viruses being detected.

Notwithstanding the limitations of sample sizes in certain studies, available data collected at sites where digested sludge has been applied to land support the conclusion that viral contamination of either surface or groundwater would be a sporadic occurrence. Unfortunately, these monitoring efforts do not provide any information on the factors that affect viral movement in sludge-amended soil. As with survival, most definitive data come from studies in which viruses have been introduced into the sludge-soil system.

With one exception, leachates from field lysimeters 125 cm in depth and seeded with Coxsackievirus B3 in sludge yielded no viruses by either direct analysis or concentration of percolates (Damgaard-Larsen et al, 1977). The single positive water sample collected from a sandy soil within 3 weeks of sludge application was attributed to possible contamination during handling. Furthermore, based on the detection of radioactively labelled water (^3H_2O) applied with sludge to two lysimeters, water did not penetrate the depth of the clay soil lysimeter and required approximately 3 months to move through 125 cm of sand. Thus, it appears that viruses were effectively retained within these sludge-amended soils under natural environmental conditions in which 30 cm of precipitation were recorded over the 6 month study period.

Using laboratory columns (7 cm diameter x 13 cm length) packed with two soil types, Moore and associates (1978) compared the transport of poliovirus applied in either secondary effluent or anaerobically digested sludge. Secondary sludge was aerated in the presence of poliovirus, subjected to high rate anaerobic digestion, dewatered on sand beds overnight and injected about 2.5 cm below the soil surface. Cyclic application of effluent and distilled water was conducted and column leachates were analyzed for viruses. Viral release was observed in both columns as conductivity of leachate decreased. However, significantly fewer virions moved through an agricultural soil when sludge was incorporated into the soil matrix. Only 0.1 to 0.2% of the total applied viruses were recovered from the percolates of sludged columns, while 47% of the viruses applied in effluent were recovered under identical conditions of elution. Similar results were obtained in a sandy loam soil with 32% of applied poliovirus detected in leachates of effluent irrigated columns and only 2% recovered in percolates through the sludge-amended sand. It should be noted, however, that water and/or effluent was constantly applied to the columns, a situation highly unlikely to be duplicated under field situations.

In another laboratory-based study, Bitton and co-workers (1978) studied the movement of sludge-bound poliovirus 1 (Sabin) through a sandy loam soil (14 to 27% clay) using either uniformly packed soil columns (4.8 cm diameter x 10 cm or 29 cm length) or undisturbed soil cores (5 cm diameter x 54 cm length). The soils were obtained from the West Florida Agricultural Experiment Station at Jay, Florida. Viruses were mixed with autoclaved, liquid digested sludge for 1 hour. Over a 12 hour period following this mixing, 31% to 47% of the polioviruses could be recovered as free (i.e., non-sludge associated) virus. The sludge was then either directly applied to the undisturbed cores or diluted 1:50 in 0.01N calcium chloride or distilled water and applied to packed columns. Aliquots of rainwater (pH 4.5, 24 μmho/cm) and 0.1N calcium chloride then were applied in various combinations to the sludge-amended soils to ascertain the potential for viral movement. Regardless of the elution protocols followed, a maximal level of 0.1 to 0.2% of the polioviruses originally introduced onto the soil were recovered in column or core percolates. While the use of divalent calcium may have confounded viral adsorption and elution, several experiments using only seeded digested sludge and rainwater yielded comparable results. Notably, similar low levels of viruses were detected when poliovirus was applied to the same soil columns in 0.01N calcium chloride followed by rainwater addition. Obviously, neither free nor sludge-associated viruses penetrated significantly through even 10 cm of this high clay soil under the elution protocols used. Bitton and associates (1978) also stated that a similar application of virus-seeded sludge to a fine sandy soil (3.2% clay) and elution with rainwater resulted in the breakthrough of more than 29% of the input virus.

The actual depth to which viruses could penetrate the soil profile was investigated in column studies using agricultural soil obtained at the Fulton County, Illinois land reclamation site (Lue-Hing et al, 1979; Metropolitan Sanitary District of Greater Chicago and IIT Research Institute, 1979). Poliovirus 1 and echovirus 7 were seeded into digested sludge and applied to prewetted columns. After 5 days of distilled water addition at a rate of 100 ml per day, representative columns were dismantled and the soils were sampled for viruses at depths from 1 cm to 20 cm. No viruses were detected in any of the five daily column percolates. Furthermore, all infectious viruses were detected within the top 1.3 cm soil sample. No viruses were recovered from a column depth of 2.6 cm or below.

As part of the Metropolitan Seattle study described under bacterial transport, indigenous viruses were also monitored in percolates from soil cores receiving sludge (Andrew Heyward, personal communication, 1983). Based on the recovery of 7 pfu/100 g of dewatered sludge at the initiation of the study, each of three test cores received about 520 viruses. Two viral plaques were recovered from the percolate of one of three test cores after the first and second cycle of water application. Thus, less than 0.4% of the viruses applied in sludge were detected moving through 46 cm of this forest soil.

The vertical transport of poliovirus 1 (LSc) and echovirus 1 (Farouk) from sludges applied to a fine sand soil has also been tested in a Florida study (Bitton et al, 1984). After seeding with the test viruses, the lagooned sludge was applied to the surface of undisturbed soil cores (16 cm diameter x 33 cm length), air-dryed, and mixed to a depth of 2.5 cm. Cores were exposed to prevailing environmental conditions, and percolates were collected in conjunction with rainfall events. Over both the summer (cumulative rainfall, 51 cm) and fall-winter (cumulative rainfall, 25 cm) monitoring periods, no viruses were detected in column leachate concentrates. However, in no leachate collection period was the equivalent of a pore volume collected. In addition, ceramic cups (1.4 to 2.1 μm pore diameter) were installed at the bottom of each column and could have facilitated the removal of any viruses in core percolate with leachate conductivity ranging from 100 to 1360 μS/cm. Indeed, even under the best test conditions, Damgaard-Larsen and associates (1977) reported minimally a 2 $\log_{10}$ loss of virus when sampled through a ceramic lysimeter.

Parasites

Only one published report addressed the potential horizontal transport of parasitic ova (Theis et al, 1978). Samples of irrigation return flow collected at the Oakland-East Bay, California soil enrichment program site were analyzed for their parasite content. Approximately 2 to 3 liter samples were quiescently held allowing soil to settle. Water was siphoned off and the remaining sediment was processed by standard zinc sulfate flotation or formalin concentration procedures. Of 38 samples examined, just over 10% were positive for ova. No controls from similar unsludged fields were reported.

Several studies have investigated the possibility of vertical movement of parasitic ova by sampling sludged fields at increasing soil depths. Since most agricultural soils are routinely tilled after sludge application, the mechanical displacement of ova to deeper depths and the disruption of the soil's integrity can complicate the interpretation of the observed results. In special studies conducted in Ontario, Canada, core samples were taken to a maximum depth of 6 cm in all fields receiving *Ascaris* ova-seeded sludge (Graham, 1981). With one exception, ascarid ova were recovered only from the top 2 cm in both pasture and bare soil plots receiving surface application of sludge. In tilled plots, ova were recovered at the 2 to 4 cm depth generally for no more than 15 days even though eggs were still present in the 0 to 2 cm sample. It should be noted, however, that sampling periods extended to only 22 to 98 days after sludge application. In addition, no precipitation data were presented.

As mentioned previously, Bergstrom and Langeland (1981) reported an increasing number of ascarid ova at soil depths below 3 cm, which correlated with the loss of eggs from the surface soil. The extent to which the experimental design may have contributed to these results, however, should be considered. Briefly, plots measuring 10 cm x 80 cm x 25 cm depth were filled with a mixture of humus, sewage sludge, and pig feces carrying Ascaris ova. Thus, the ova were added independently of the sludge. Perhaps more importantly, the soil would have lacked structural integrity and extensive settling and compaction may have occurred as the study progressed. Seven weeks into the experiment, greater numbers of eggs were recovered from depths of 6 to 12 cm and 12 to 25 cm than at 3 to 6 cm. While it is conceivable that very rapid displacement through the soil profile occurred as a result, perhaps, of heavy rain, a stronger argument for vertical movement could be made if a gradient of ova had been recovered showing progressive movement with increasing depth.

Notably, at an actual sludge disposal site near Calgary, Alberta, Canada, Lehmann and associates (1983) evaluated the vertical distribution of parasitic ova in fields sludged from 1978 to 1980. As mentioned previously, parasite eggs were recovered throughout the 15 cm profile sampled; however, most eggs (68% to 85% of the total recovered) were detected at 0 to 2.5 cm while less than 3% of all ova recovered came from the 10 to 15 cm depth.

Preliminary data collected in 1984 during an ongoing study of Ascaris ova survival in sludge-amended soils provide good evidence for ova retention within the upper portion of a clay soil profile (Walter Jakubowski, personal communication, 1984). At a Cincinnati test site, seeded sludge was tilled into the test plot in June resulting in ova concentrations of about 150 eggs/g of soil in the top 10 cm of soil sampled in July. The field was left fallow through the remainder of the year. In two subsequent samples collected in September and November, ova concentrations at 0 to 10 cm remained around 150/g and no substantial increase was observed in the number of Ascaris ova recovered from a depth of 10 to 20 cm (less than 20/g). Monthly precipitation totals ranged from about 6.7 cm to 12 cm with cumulative rainfall plus irrigation reaching about 54 cm from June through November. Prior to spring cropping at the New Orleans and Lubbock sites, more ova were recovered from surface (0 to 10 cm) samples than from the 10 to 20 cm depth. Thereafter, inactivation of ova at the surface and tilling events make conclusions regarding transport difficult.

SECTION 8

DISCUSSION

SURVIVAL OF MICROORGANISMS IN SLUDGE-AMENDED SOILS

Drawing upon available literature, Gerba and associates (1975) summarized the factors affecting microbial survival in soil as soil moisture content, temperature, pH, sunlight, organic matter, and antagonistic soil microflora. Microbial pathogens introduced into the soil by sludge amendment will be influenced by these factors. However, the nature of the sludged soil environment may moderate soil conditions in ways that could impact the survival of microorganisms. For example, sludge application may dramatically increase the organic content, nutrient content, and moisture retention capability of sandy soils. Additionally, soil pH could be influenced by added sludge or management practices such as liming. Even soil temperature could be affected by the surface application of sludge. While the interplay of these changes in sludge-amended soil may favor organism survival in some cases, in other situations more rapid pathogen inactivation may occur.

Certainly those environmental conditions favorable to bacterial survival would be those allowing organism growth, e.g., availability of nutrients and optimal soil moisture and temperature. While not promoting growth, low temperatures could contribute to the extended persistence of bacteria by slowing metabolic activity. For example, *Salmonella typhi* has been recovered from loam and peat soils for periods up to 85 days, while survival in drying sand was 4 to 7 days (Beard, 1938; Beard, 1940). Additionally, *S. typhi* may survive as long as 2 years at freezing temperatures.

Available data on the recovery of *Salmonella* sp. from sludge-amended soil illustrates some of the factors which can influence bacterial survival. As shown in Table 16, T_{90} values for *Salmonella* fall within the range of 3 to 61 days with median values of 12 days and 8 days for soil depths of 0 to 5 cm and 5 to 15 cm, respectively. Notably, the inclusion of data (9 observations) from a single study by Dickson and Tribe (1986) influenced the median T_{90} values at the greater soil depth. Excluding these data, a median T_{90} for *Salmonella* at 5 to 15 cm depth was 15 days. Reviewing selected studies more closely, a seasonal trend of bacterial inactivation can be seen. When *Salmonellae* in sludge-amended soils were subjected to winter conditions, T_{90} values of 12 days to 15 days (Kenner et al, 1971), 17 days (Andrews et al, 1983), 22 days and 61 days (Jones et al, 1983) have been estimated. Similarly, at a temperature of 12°C, *Salmonella* sp. were observed to decay with a T_{90} of 8 to 11 days in a controlled laboratory study (Dickson and Tribe, 1986). During summer exposure a much more rapid inactivation of indigenous *Salmonellae* has been observed with T_{90} values of 4 days (Andrews et al, 1983)

TABLE 16. SUMMARY OF MICROORGANISM SURVIVAL IN SLUDGE APPLIED TO SOIL

Bacteria	Depth (cm)	Die-off -- T_{90} (days)				Die-off -- T_{99} (days)			
		Minimum	Maximum	Median	Observations	Minimum	Maximum	Median	Observations
Salmonellae	0 - 5	6	61	12	10	11	45	22	8
	5 - 15	3	22	8	17 +	7	45	18	15 +
Fecal Streptococci	0 - 5	7	28	14	9	14	63	24	8
	5 - 15	12	30	20	11 +	30	60	40	10 +
Fecal Coliforms	0 - 5	7	84	25	19	12	165	53	16
	5 - 15	4	49	13	12	9	90	32	11
Total Coliforms	0 - 5	16	172	85	6	28	350	155	4
	5 - 15	9	70	17	12 +	18	40	32	9 +
Viruses	0 - 5	< 1	30	3	9	2	52	6	6
	5 - 15	12	56	30	4	60	100	60	3
Parasites	0 - 5	17	270	77	11	68	500	81	5
	5 - 15		Data unavailable				Data unavailable		

+ Includes data not shown in Tables 10, 11 and 13, taken from Dickson and Tribe (1986).

and 6 days (Jones et al, 1983). Studies conducted during warm growing seasons in which laboratory grown strains of Salmonellae were employed have resulted in T_{90} values of 6 days and 10 days (Larkin et al, 1978) and 14 days (Bergstrom and Langeland, 1981). Hence, studies with both indigenous and seeded Salmonellae are in relatively good agreement showing, with one exception, a 90% bacterial reduction within 3 weeks of sludge application.

Results of studies in which cattle grazing on sludged forage have been monitored for salmonellosis also suggest a relatively short survival time for Salmonella. Based on literature in which time courses can be ascertained, animal infections have occurred when exposure was within 1 month of sludge application. While some Salmonellae are undoubtedly still viable beyond 1 month, these observations suggest that die-off to a level below the infectious dose had occurred within this time frame.

The exceptionally long survival times often cited for Salmonella persistence actually arise from seeded studies in which high levels of bacteria ranging from 10^6 to 10^{10} per liter were added to sludge prior to its land application. Under these conditions and assuming a maximum T_{99} of 45 days, persistence times in excess of 5 months could be anticipated. If growth or regrowth of seeded organisms occurs, this survival time could be substantially longer. At actual field sites, indigenous Salmonellae have generally persisted at low levels for less than 2 months although positive recoveries reflecting low levels of bacteria have been made as long as 3 to 5 months after sludge application.

Indicator bacteria display a wide range of T_{90} values, although the behavior of fecal streptococci and fecal coliforms are reasonably similar when comparing median values (Table 16). With the exception of one study in which high loading rates were used (Edmonds, 1976), 90% of the fecal coliforms could not be recovered within 6 weeks of sludge application. Fecal streptococci displayed a comparable loss within 4 weeks. Although the number of studies is more limited, total coliform bacteria displayed slower inactivation in sludge-amended soils. It should be noted, however, that T_{90} values for total coliforms are polarized with most values ranging from 14 to 42 days and a second group ranging from 129 to 172 days. In evaluating the overall survival results of these groups of bacteria, a dichotomy exists with the seasonal survival of coliform bacteria as reported in two studies conducted in the Pacific Northwest region of the United States. Although the actual T_{90} values were dramatically different, both Edmonds (1976) and Hagedorn (1980) observed longer survival times during warmer months than during cooler months. Coliform growth would be the most likely explanation of these findings. Indeed, Edmonds (1976) noted the regrowth of coliform bacteria in the spring following a decrease in levels during the winter months. These results highlight the difficulty in evaluating bacterial inactivation when organisms are capable of replication. Interpretation of data for indicator bacteria is further complicated by the fact that several bacterial species including unique soil microflora may be recovered by the analytical procedures used. These bacterial populations do not necessarily share the same inactivation or regrowth characteristics.

Survival of viruses in soils is influenced by many of the same parameters described above, although at this time little direct evidence supports viral inactivation by antagonistic microorganisms. The effect of temperature on the survival of viruses is well documented. Lower temperatures favor longer survival times. In one study, observation was made of a T_{90} of approximately 3 months at 4°C for poliovirus, 1 month at 20°C, and less than 1 week at 30°C (Duboise et al, 1976). Further, an optimal soil moisture content favors poliovirus survival in soil, while desiccation results in a more rapid loss of viruses. It should also be remembered that, as obligate intracellular parasites, viruses do not replicate outside of an appropriate host. Thus, their numbers eventually decrease in a soil environment.

Data characterizing the survival of viruses in sludge-amended soil are perhaps more straightforward. T_{90} values have ranged from less than 1 day under hot summer conditions (Tierney et al, 1977; Farrah et al, 1981) to 56 days in the winter (Damgaard-Larsen et al, 1977). It should be noted that data presented in Table 16, which appear to support extended viral survival with depth, reflect sampling to a depth of 20 cm (3 observations) in one Danish study where an average temperature of 0.5°C was determined (Damgaard-Larsen et al, 1977).

Undoubtedly, viruses can persist for extended periods of time in the soil environment where cold temperatures favor their survival. Studies completed under winter conditions in both Denmark (Damgaard-Larsen et al, 1977) and Ohio (Tierney et al, 1977) showed very similar inactivation with T_{90} values of 30 days for two different enteric viruses. As evidenced by available data, however, inactivation at the soil surface can be quite rapid when viruses are exposed to higher temperatures and drying conditions such as those described in Texas (Hurst et al, 1978) and Florida (Farrah et al, 1981; Bitton et al, 1984) in the summer and fall.

Among the parasites, protozoa seem to be very sensitive to drying and under these conditions survival is usually short. Kowal (1983) suggested maximum survival times for protozoa in soil to be 10 days, with 2 days being more common. As stated in the RESULTS section, ova of helminths such as *Ascaris* are quite resistant to environmental stress and extended persistence times of up to 15 years have been reported (Krasnonos, 1978; and Jackson et al, 1978). However, as with all other microbial pathogens, desiccation can reduce survival substantially (Reimers et al, 1980) and a more practical maximum survival time appears to be 2 years (Kowal, 1983). Parasites are also unable to replicate outside of their appropriate animal or human hosts.

Only three independent research groups have reported quantitative data which allow estimation of parasite inactivation in sludge-amended soil. All but one T_{90} value are based upon the addition of exogenous *Ascaris* or *Toxocara* ova into the sludge-soil system. As expected, T_{90} values for these parasitic forms exceeded those of all other microbial groups, ranging from 17 days to 270 days with a median value of 77 days (Table 16). Seasonal effects upon ova survival were observed. Following summer sludge application on bare soil, *Ascaris* ova were inactivated with an apparent T_{90} value of 17 days.

Survival after applying sludge in the fall was more extended with a T_{90} value of 6[illegible] days for Ascaris ova and 77 days for Toxocara ova (Graham, 1981). Similarly, a T_{90} value of 30 days was estimated for Ascaris in sludge sprayed onto an untilled plot in the summer, while 90% inactivation following winter application required 80 days and 90 days (Walter Jakubowski, personal communication, 1984). Notably, after winter sludge application, Ascaris ova survived dramatically longer with a T_{90} of 200 days in tilled plots planted with a cover crop in the spring (Walter Jakubowski, personal communication, 1984). Presumably, decreased soil temperature due to crop shading and higher soil moisture due to irrigation and rainfall favored this extended survival time. This research program evaluating Ascaris ova survival at field sites in Ohio, Texas, and Louisiana is continuing and may provide valuable insights into factors affecting the survival of this parasite in sludge-amended soils.

Attempts were made to statistically analyze the quantitative data presented in Tables 10 through 15. Unfortunately, sufficient data were available only for temperature and die-off.

Least-squares regression analyses of raw and transformed data were performed for the die-off rates recorded for Salmonellae (Figures 2 and 3), fecal streptococci (Figure 4), fecal coliforms at 0 to 5 cm depth (Figure 5), fecal coliforms at 5 to 15 cm depth (Figure 6), total coliforms (Figure 7), viruses (Figure 8), and parasites (Figure 9). Only for fecal coliforms were there sufficient data to discriminate between depths. In the cases of Salmonellae, viruses, and parasites, die-off at all depths was used in the analysis. Die-off at 5 to 15 cm was used in the analysis of Salmonellae, fecal streptococci, and total coliforms.

The equations of the regression lines and the correlation coefficient for each data set are shown in Table 17. Poor correlation was observed for Salmonellae, fecal streptococci, total coliforms, and parasites while very good correlation was observed for fecal coliforms at both depths and for viruses. More often than not, the transformed T_{90}/T_{99} data correlated better with temperature but the difference was not judged to be significant.

One major anomaly was observed in these data. The survival of fecal coliforms at depths of 5 to 15 cm increased with increasing temperature. The most plausible explanation of this observation is organism growth in the enriched environment of the sludge-soil matrix. However, caution must be exercised in generalizing this interpretation, since all data used in these statistical analyses for fecal coliforms at the 5 to 15 cm depth were collected in a single study conducted in Oregon (Hagedorn, 1980).

Close scrutiny of these data shows that usable information for both fecal coliforms and viruses was available only at temperature extremes. Or to put it another way, data tended to be clumped at either the warm or cold ranges with little data in between. This observation restricts the value of an analysis over a range of temperatures and suggests the need for more detailed evaluation.

One approach to this evaluation is to use nonparametric correlations which make no assumptions about the normality of the distribution of the

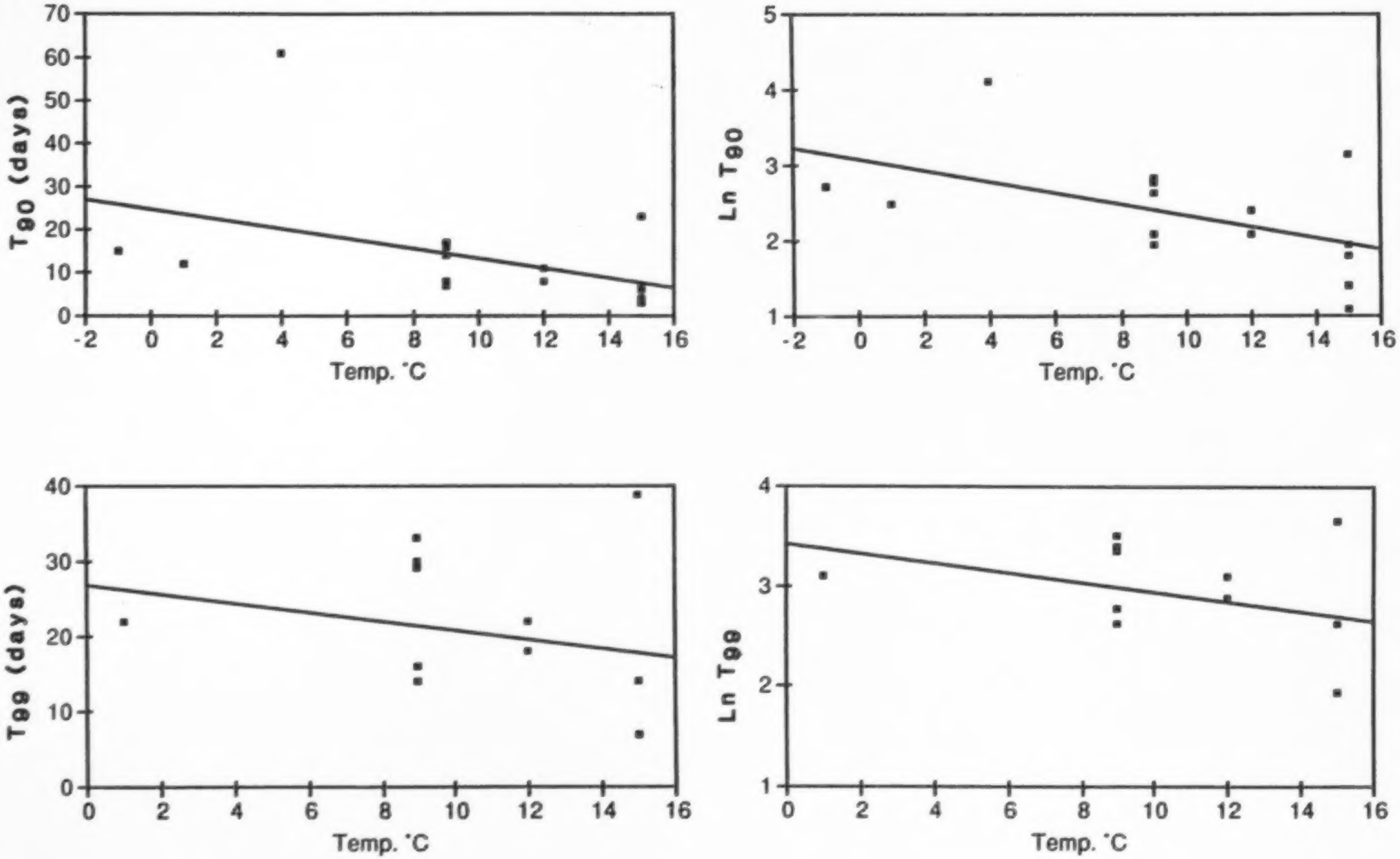

Figure 2. Least Squares Regression Plots for Temperature and Salmonella Survival at all Depths.

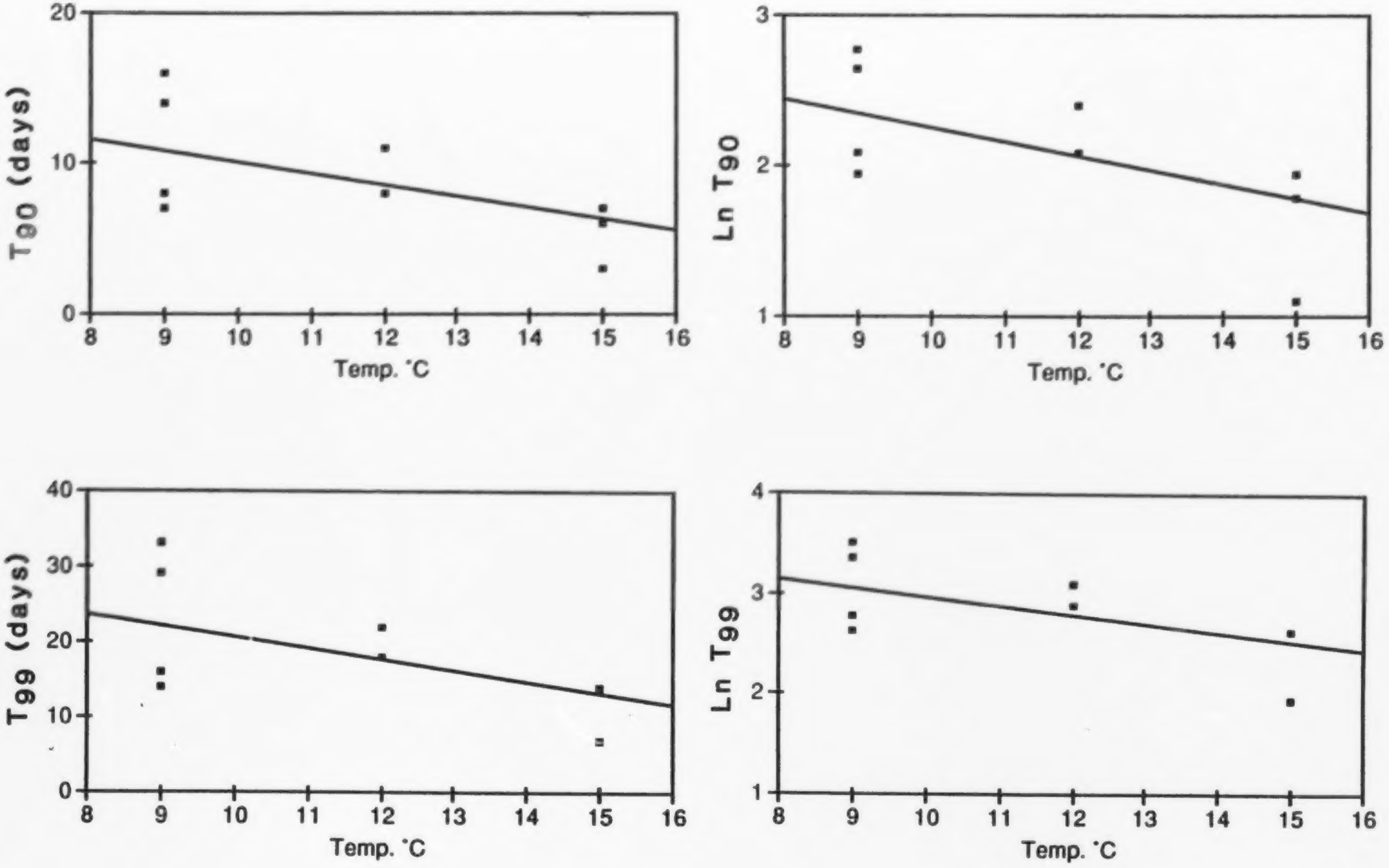

Figure 3. Least Squares Regression Plots for Temperature and Salmonella Survival at 5-15cm Depths.

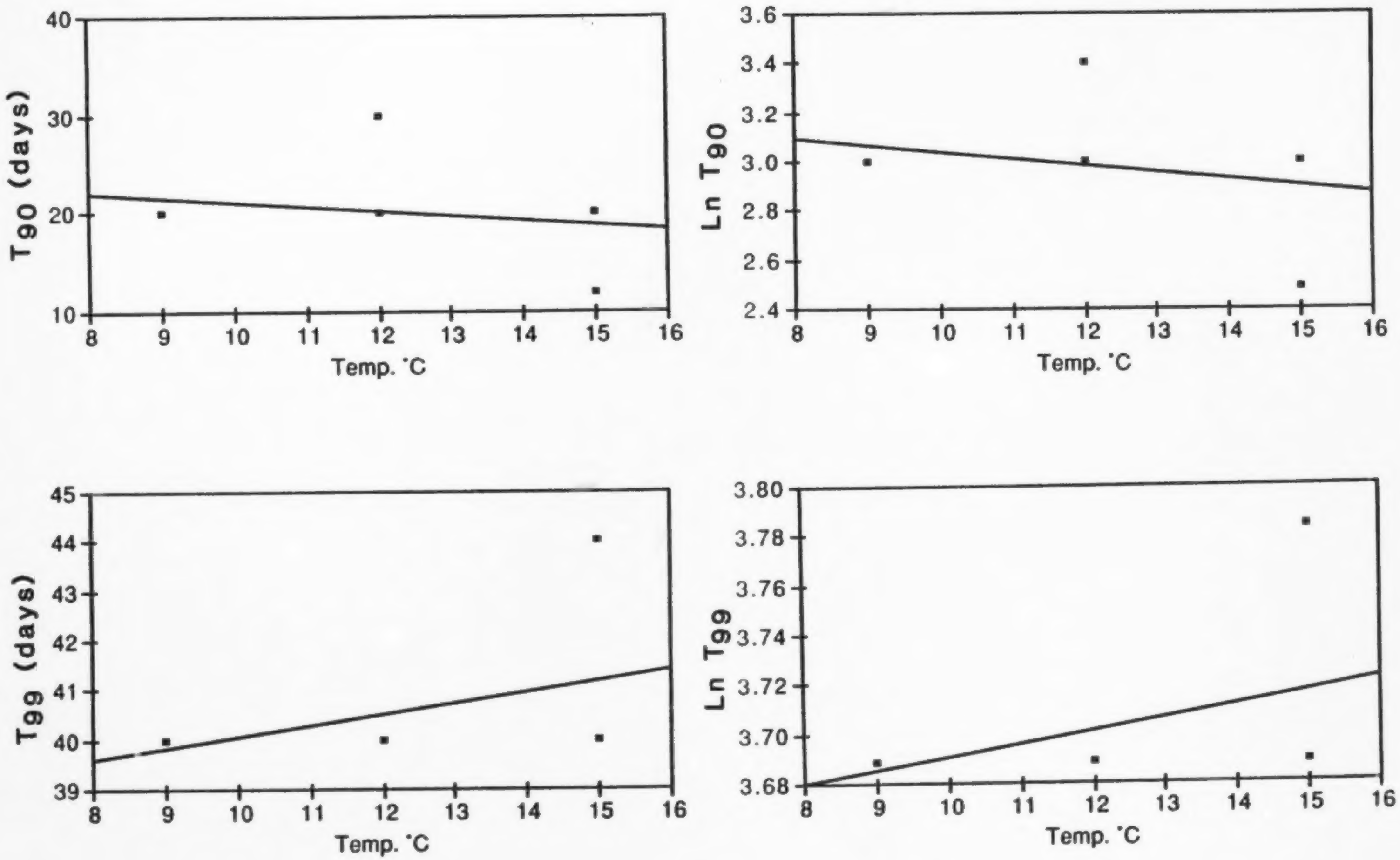

Figure 4. Least Squares Regression Plots for Temperature and Fecal Streptococcus Survival at 5-15cm Depths.

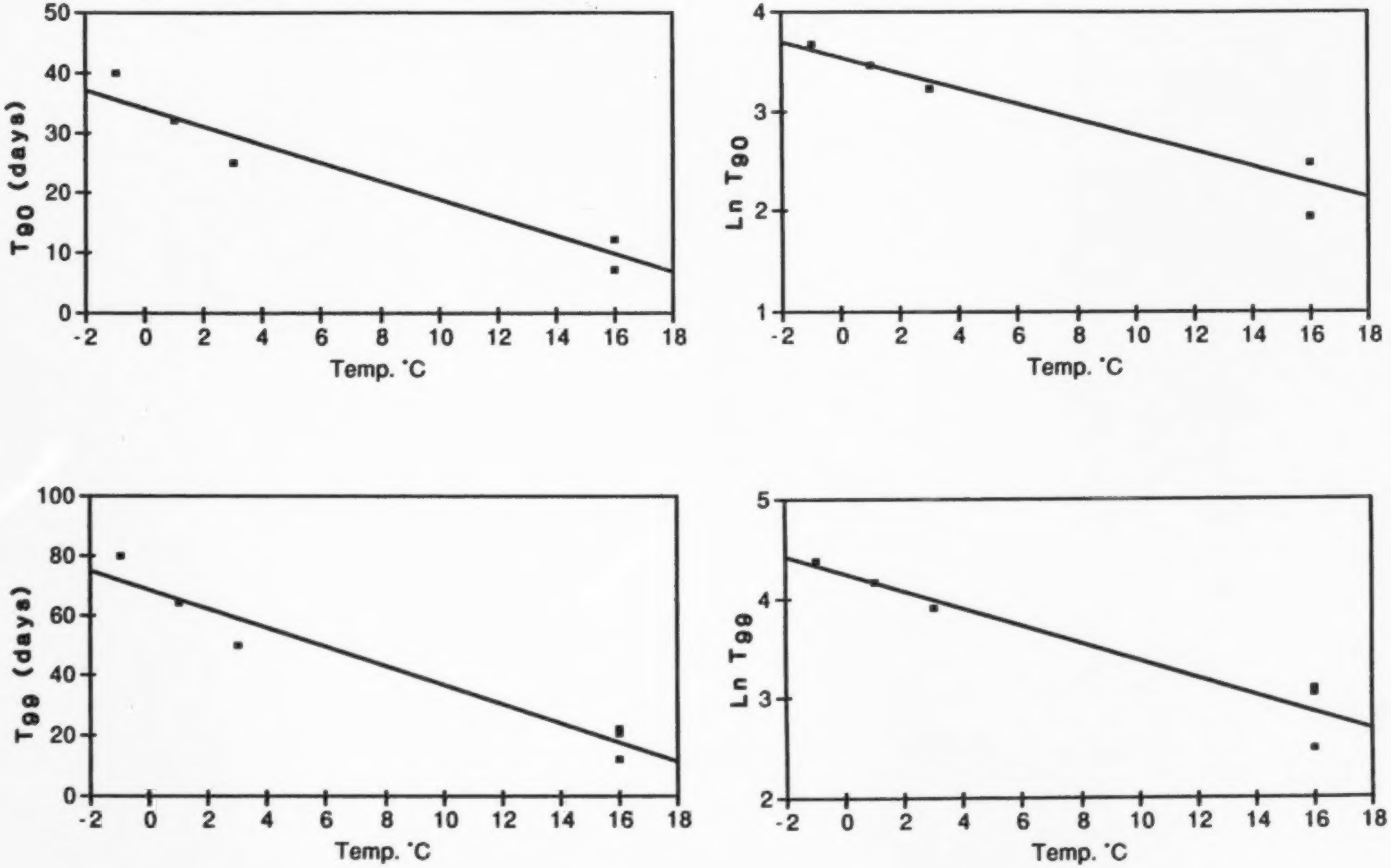

Figure 5. Least Squares Regression Plots for Temperature and Fecal Coliform Survival at 0-5cm Depths.

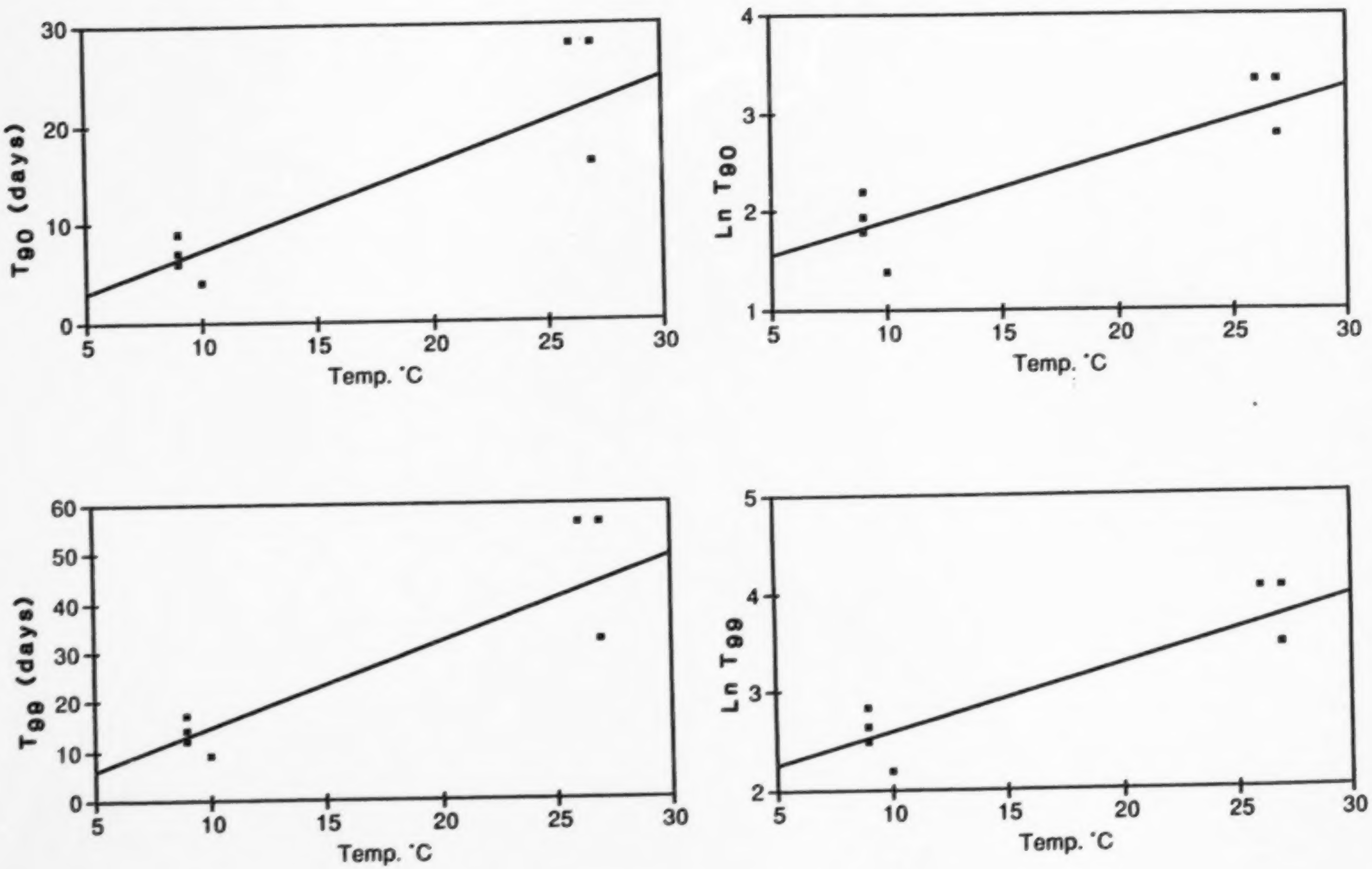

Figure 6. Least Squares Regression Plots for Soil Temperature and Fecal Coliform Survival at 5–15cm Depths.

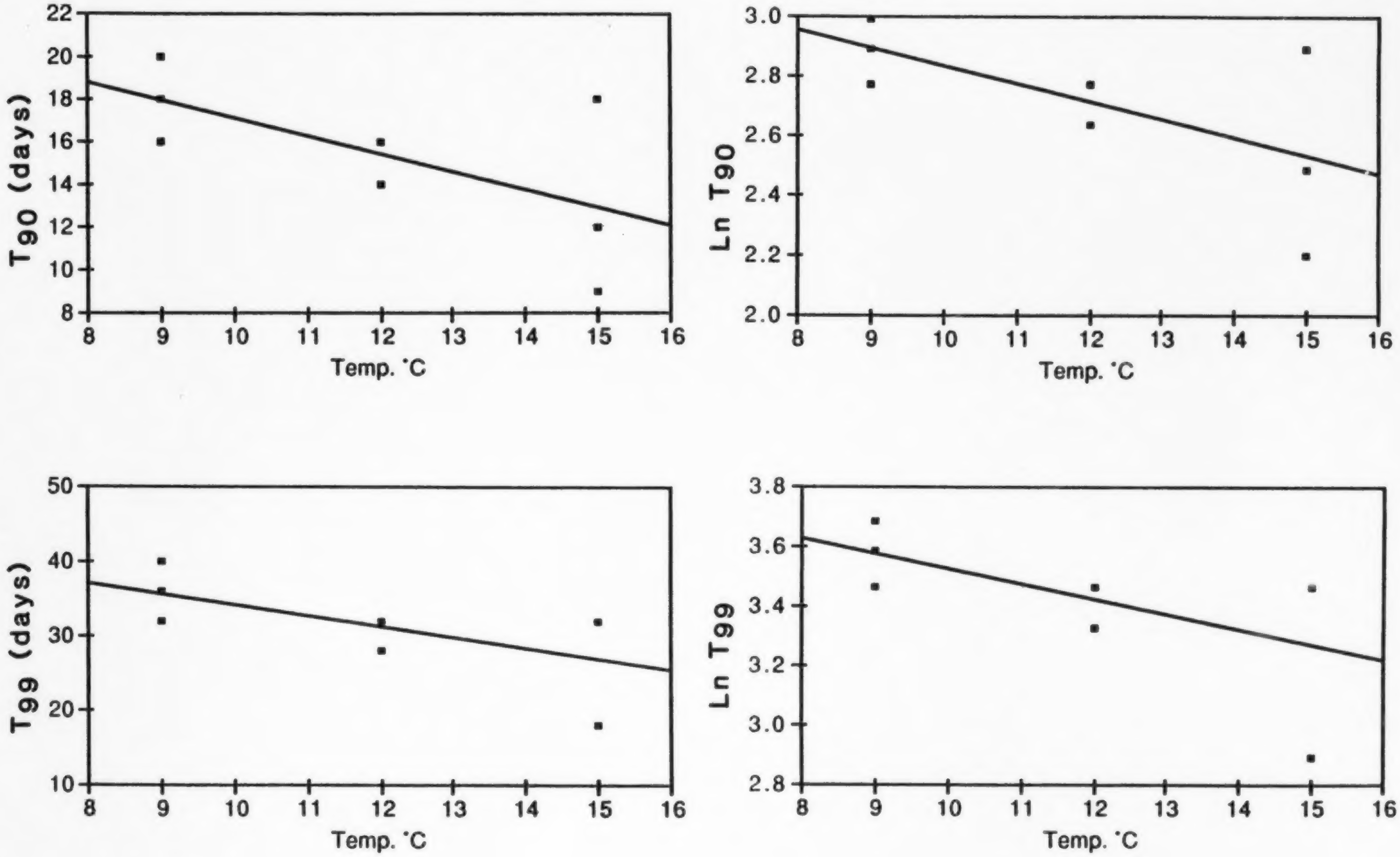

Figure 7. Least Squares Regression Plots for Soil Temperature and Total Coliform Survival at 5-15cm Depths.

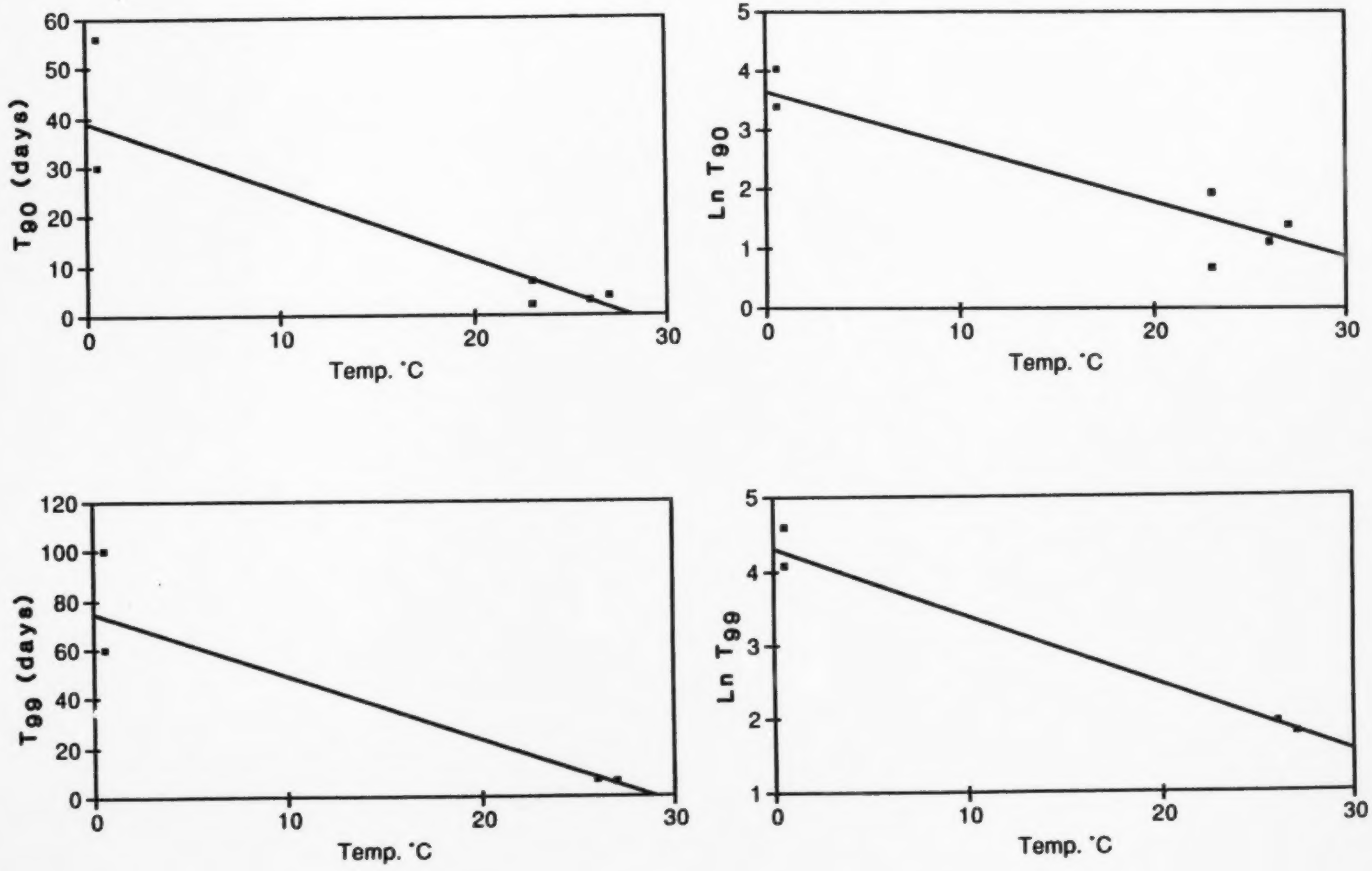

Figure 8. Least Squares Regression Plots for Temperature and Virus Survival at all Depths.

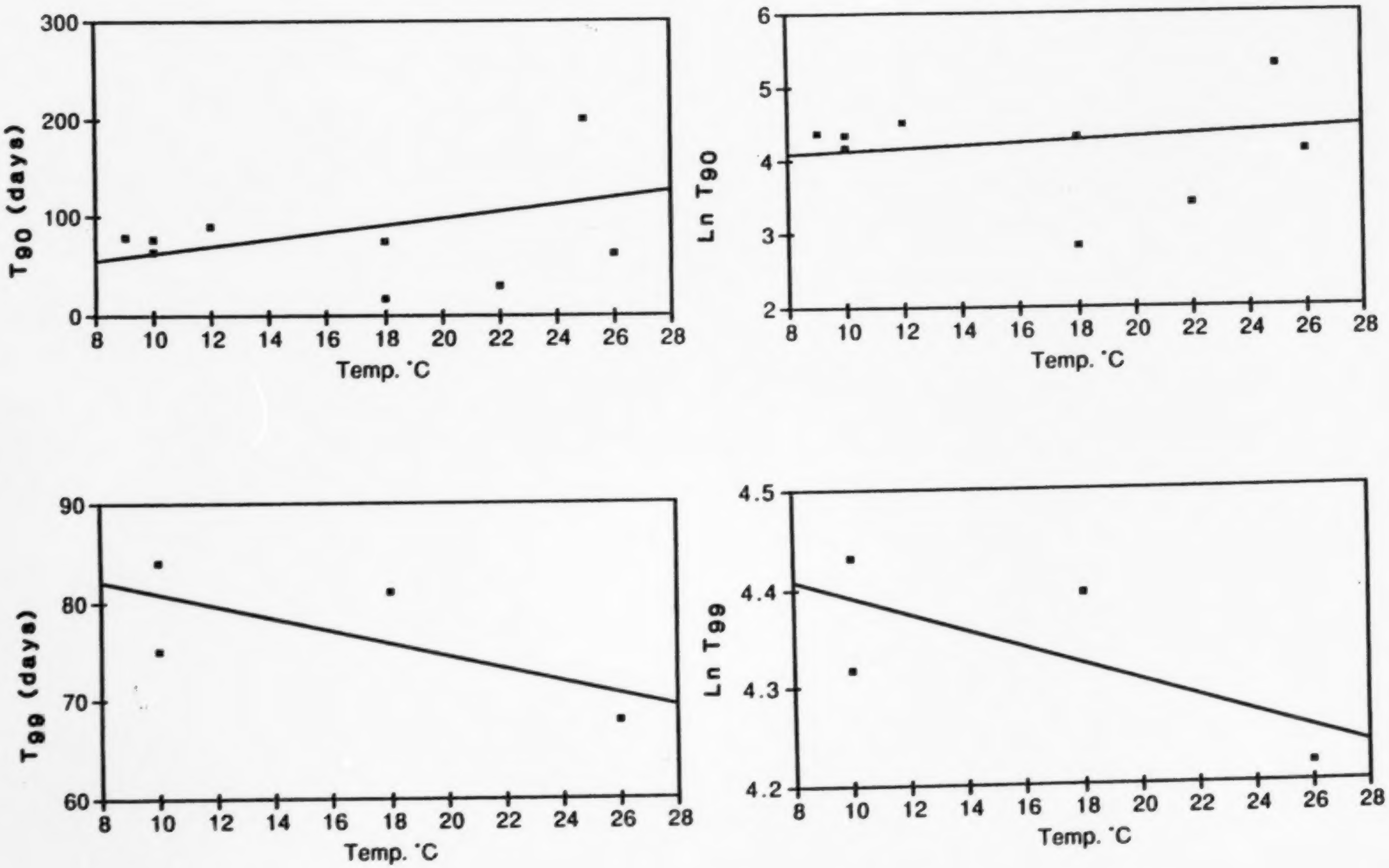

Figure 9. Least Squares Regression Plots for Temperature and Parasite Survival at all Depths.

TABLE 17. SUMMARY OF STATISTICAL ANALYSES FOR MICROBIAL DIE-OFF RATES AND TEMPERATURE

Organism	Depth (cm)	Regression Equation (T_{90}/T_{99} in days; t=°C)	Correlation Coefficient (r)	Kendall Rank Order Correlation Coefficient (p)	Kendall Rank Order Correlation Significant at 95% Level
Salmonellae	All	$T_{90} = 26.13 - 0.87\ t$	0.318	0.392	No
		$\ln T_{90} = 3.12 - 0.05\ t$	0.412		
		$T_{99} = 27.15 - 0.05\ t$	0.023	0.079	No
		$\ln T_{99} = 3.43 - 0.03\ t$	0.230		
	5 - 15	$T_{90} = 17.65 - 0.76\ t$	0.565	0.384	No
		$\ln T_{90} = 3.19 - 0.09\ t$	0.579		
		$T_{99} = 35.50 - 1.49\ t$	0.519	0.399	No
		$\ln T_{99} = 3.85 - 0.09\ t$	0.543		
Fecal Streptococci	5 - 15	$T_{90} = 25.56 - 0.44\ t$	0.255	0.382	No
		$\ln T_{90} = 3.33 - 0.03\ t$	0.320		
		$T_{99} = 37.83 + 0.22\ t$	0.436	0.001	Yes
		$\ln T_{99} = 3.64 + 0.01\ t$	0.436		
Fecal Coliforms	0 - 5	$T_{90} = 34.25 - 1.52\ t$	0.966	0.043	Yes
		$\ln T_{90} = 3.54 - 0.08\ t$	0.954		
		$T_{99} = 68.62 - 3.19\ t$	0.971	0.045	Yes
		$\ln T_{99} = 4.24 - 0.09\ t$	0.957		
	5 - 15	$T_{90} = -1.41 + 0.87\ t$	0.854	0.020	Yes
		$\ln T_{99} = 1.21 + 0.07\ t$	0.889		
		$T_{99} = -2.84 + 1.74\ t$	0.857	0.020	Yes
		$\ln T_{99} = 1.92 + 0.07\ t$	0.905		

TABLE 17. SUMMARY OF STATISTICAL ANALYSES FOR MICROBIAL DIE-OFF RATES AND TEMPERATURE (Continued)

Organism	Depth (cm)	Regression Equation (T_{90}/T_{99} in days; t=°C)	Correlation Coefficient (r)	Kendall Rank Order Correlation Coefficient (p)	Kendall Rank Order Correlation Significant at 95% Level
Total Coliforms	5 - 15	$T_{90} = 25.44 - 0.83\ t$	0.645	0.252	No
		$\ln T_{90} = 3.44 - 0.06\ t$	0.642		
		$T_{99} = 48.67 - 1.44\ t$	0.625	0.116	No
		$\ln T_{99} = 4.04 - 0.05\ t$	0.593		
Viruses	All	$T_{90} = 39.09 - 1.39\ t$	0.906	0.149	No
		$\ln T_{90} = 3.63 - 0.09\ t$	0.942		
		$T_{99} = 74.61 - 2.57\ t$	0.913	0.127	No
		$\ln T_{99} = 4.31 - 0.09\ t$	0.987		
Parasites	All	$T_{90} = 28.43 + 3.49\ t$	0.382	0.393	No
		$\ln T_{90} = 3.95 + 0.02\ t$	0.164		
		$T_{99} = 87.18 - 0.64\ t$	0.689	0.235	No
		$\ln T_{99} = 4.48 - 0.01\ t$	0.700		

variables. The Kendall rank-order correlation was chosen for this evaluation (Snedecor, 1956). Calculated significances for data sets are shown in Table 17. Of those data which had a high correlation with temperature, only fecal coliform data at both the 0 to 5 cm depth and the 5 to 15 cm depth were judged to be significant at the 5 percent level.

TRANSPORT OF MICROORGANISMS FROM SLUDGE-AMENDED SOILS

In addition to survival of pathogens in sludge-soil systems, consideration must be given to their ability to move in this environment, either into surface waters via runoff or, perhaps more importantly, to groundwater through the soil profile. While runoff may be viewed as largely the physical transport of microorganisms associated with particulate material, vertical microbial transport is more complex.

Exceedingly few studies have addressed the presence of microorganisms in runoff from sludge-amended soils. No significant bacterial or viral impact has been observed on surface water at sludge application sites (Lue-Hing et al, 1979; Edmonds, 1976). However, as long as viable bacteria were present in sludge-amended soil, they were recovered at elevated levels from runoff intercepted at the lower end of sludged fields (Dunigan and Dick, 1980). Similarly, parasitic ova have been recovered from irrigation return flow at a sludge application site (Theis et al, 1978). Obviously, sludge application methods that minimize the displacement of sludge in surface runoff should be used if microorganism transport in runoff is to be avoided.

Removal of bacteria from wastewater percolating through a soil is due to both mechanical action (i.e., straining or sieving at the soil surface) and adsorption to soil particles. Bacterial movement through soils has been demonstrated at several field sites. Reporting from the available literature, Gerba and coworkers (1975) noted movement of bacteria in a variety of soils for distances ranging from 0.9 to 456 m. Release and movement of microorganisms would be expected, since physical adsorption to particulates is a reversible phenomenon and, in part, ion dependent.

The phenomenon of adsorption as a mechanism for the retention of viruses in soil systems also has been demonstrated by a number of investigators (Duboise et al, 1976; Lance et al, 1976; and Funderburg et al, 1981). In general, virus adsorption by soils increases with increasing ion-exchange capacity, clay content and organic carbon. Viral release from soils may be correlated with decreasing conductivity of soil water (Duboise et al, 1976; Funderburg et al, 1981). Movement of viruses through soils in field studies has been demonstrated to depths of 67 m (Keswick, 1984).

The transport of protozoa and helminths in soils appears to be more limited than for bacteria or viruses (Metro, 1983a). This may be the result of the considerable size difference between viruses, bacteria, and parasites (see Table 18). For example, protozoa are up to 20 times larger than bacteria and up to 2000 times larger than enteroviruses. Ascaris eggs are even larger. Clearly, mechanical straining may be the most important factor governing parasite transport.

TABLE 18. SIZES OF SELECTED VIRUSES, BACTERIA, AND PARASITES (After Bitton and Gerba, 1984)

Microorganism	Size (µm)
Viruses	0.02 - 0.08
Hepatitis A	
Rotavirus	
Norwalk-like agents	
Bacteria	1 - 10
Salmonella sp.	
Shigella sp.	
Vibrio cholerae	
Protozoans (cysts)	5 - 20
Giardia lamblia	
Helminths (eggs)	35 - 50
Ascaris sp.	45 - 75

Relatively few studies conducted at sludge application sites have looked for vertical transport of microorganisms. Limited monitoring has shown no demonstrable impact of sludge application on fecal coliform levels in groundwater (Edmonds, 1976; Lue-Hing et al, 1979). However, coliform bacteria have been detected at shallow depths of 100 cm (Hagedorn, 1980) and 180 cm (Edmonds, 1976) beneath sludged sites in the northwestern United States. In contrast, viruses have been recovered from deep wells (8.5 m and 18 m) at one sludge disposal site in Florida (Wellings et al, 1978) while results of groundwater monitoring at a second location in Florida were negative for viruses (Farrah et al, 1981). Comparison of such results at operational field sites is often impeded, however, by the fragmentary nature of available data. Few studies have conducted integrated, long-term monitoring for viruses in which sludge, sludged-soil and groundwater sampling were coordinated. Available literature strongly favors the contention that parasitic ova are retained at the point of sludge introduction. Groundwater monitoring for parasites has not been conducted and seems unnecessary given the relative size of most parasitic forms and their observed retention in the upper soil profiles.

Notwithstanding these limited field data, laboratory studies have demonstrated some bacterial and viral transport through sludge-amended soils. A single study has demonstrated movement of *Yersinia*, fecal coliforms and fecal streptococci through 46 cm of soil, although maximal levels represented less than 0.1% of the bacteria present in the sludge (Andrew Heyward, personal communication, 1983). It should be noted that experimental conditions in this study were chosen to represent a "worst case" situation in which a total of 46 cm of rainfall was applied over a 3 week period.

Several laboratories have studied the vertical movement of viruses in model soil columns or cores. Studies in which lysimeters or cores amended with virus-contaminated sludge have been exposed to natural rainfall have confirmed results obtained by groundwater monitoring at most sludge application sites. Specifically, virtually no viruses were detected in soil water percolates intercepted at depths of 54 cm (Bitton et al, 1978) and 125 cm (Damgaard-Larsen et al, 1977). Under certain laboratory test conditions, however, viruses applied with sludges have been transported through soil depths ranging from 13 cm (Moore et al, 1978) to 46 cm (Andrew Heyward, personal communication, 1983). When compared to the movement of "free" viruses, sludge-bound virions are much more effectively retained, apparently within the sludge-soil matrix at the point of application (Damgaard-Larsen et al, 1977; Lue-Hing et al, 1979).

RISK ASSESSMENT MODELING FOR SLUDGE-AMENDED SOILS

The objectives of this study did not include an assessment of available modeling efforts designed to evaluate the human risk associated with land application of sludge. However, the authors felt it judicious to acknowledge efforts in this area.

A recent feasibility study completed by Battelle Laboratories identified a total of four possible models which could be applied to the description of risk to humans from pathogens in sludge applied to soil (Cornaby et al, 1985). Two existing models specifically address pathogens in sludge: one was developed by BDM Corporation (Sheridan et al, 1979), and a second by Seattle Metro (Metro, 1983b). In addition, a specialized wastewater model described by Haas (1983) was judged appropriate to situations involving sludge disposal, especially in the area of risk due to potential water contamination. A fourth modeling approach was constructed by coupling three existing models (Haas, 1981; Hetrick and McDowell-Boyer, 1984; Furumoto and Mickey, 1967) to form a composite risk assessment model for pathogens in wastewater or sludge (Cornaby et al, 1985).

These models vary in their complexity and in the manner in which processes such as pathogen survival and transport to the point of human contact were considered. Nonetheless, the issue germane to this report is not the ability to model but rather whether adequate and appropriate data are available to validate such risk assessment models. Based on the review of available literature on microbial survival and transport in sludge-amended soils, insufficient data are available for this purpose. In fact, there are essentially no data available in the published literature that would permit estimating transport rates for pathogens from sludge-amended soils.

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